THE EFFECT OF VITAMIN D SUPPLEMENTATION ON CARDIOMETABOLIC RISK FACTORS AND HEALTH-RELATED QUALITY OF LIFE AMONG URBAN VITAMIN D DEFICIENT PREMENOPAUSAL WOMEN: A RANDOMISED CONTROLLED TRIAL

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ABSTRACT

Recent evidence has demonstrated that vitamin D deficiency is associated with cardiometabolic risk factors such as hypertension, diabetes mellitus, and hypercholesterolaemia, along with its pivotal role in musculoskeletal diseases. However, there remains a lack of randomised controlled trials required to establish causality between vitamin D and cardiometabolic risk factors. The aim is to determine whether Vitamin D supplementation in urban premenopausal women with vitamin D deficiency could improve cardiometabolic risk factors and health-related quality of life (HRQOL). A double-blind randomized controlled trial was conducted in Kuala Lumpur, Malaysia. A total of 192 premenopausal women were randomly selected to receive either placebo (n = 99) or vitamin D supplements of 50,000 IU once a week for two months and then 50,000 IU monthly for 10 months (n = 93). The participants were vitamin D deficient (< 50 nmol/l) at baseline. Primary outcomes were serum 25(OH)D, serum lipid profiles, blood pressure, and HOMA-IR, all of which were measured at baseline and at six and 12 months. HRQOL was assessed with SF-36 at baseline and 12 months. Ninety three and 99 women were randomised into the intervention and placebo groups, respectively. After 12 months, significant differences were found between the intervention and placebo groups in terms of serum 25(OH)D concentration (mean difference: 49.54; 95% CI: 43.94 to 55.14 nmol/l) and PTH levels (mean difference: -1.02; 95% CI: -1.67 to -0.38 pmol/l). There was no effect of vitamin D supplementation on HOMA-IR, serum lipid profiles or blood pressure (all $p > 0.05$) between the two groups. There was a small but significant improvement in the HRQOL components of vitality (mean difference: 5.041; 95% CI: 0.709 to 9.374) and mental component summary score (mean difference: 2.951; 95% CI: 0.573 to 5.329) in the intervention group compared to the placebo group. In conclusion, large and less frequent dosages of vitamin D supplementation were effective to improve vitamin D deficiency to vitamin

D sufficiency. However, there was no improvement in measured cardiometabolic risk factors in premenopausal women. On the other hand, vitamin D supplementation appeared to improve some components of HRQOL. Longitudinal studies may be needed to establish causality between vitamin D and cardiometabolic risk factors and HRQOL.

ABSTRAK

Bukti semasa menunjukkan kekurangan vitamin D dikaitkan dengan faktor-faktor risiko kardiometabolik seperti tekanan darah tinggi, kencing manis dan kolesterol tinggi selain peranan pentingnya dalam penyakit muskuloskeletal. Walau bagaimanapun, terdapat kekurangan dalam kajian rawak terkawal (randomized controlled trial) untuk membuktikan sebab dan akibat daripada vitamin D dengan faktor-faktor risiko kardiometabolik. Tujuan kajian adalah untuk menentukan sama ada Vitamin D suplemen boleh meningkatkan faktor risiko kardiometabolik dan kualiti kesihatan yang berkaitan dengan kehidupan (HRQOL) di kalangan wanita premenopausal di bandar dengan kekurangan vitamin D. Kajian rawak terkawal secara "*double–blind*" telah dijalankan di Kuala Lumpur, Malaysia. Seramai 192 wanita premenopausal telah dipilih secara rawak untuk menerima sama ada vitamin D suplemen 50,000 IU sekali seminggu selama dua bulan dan 50,000 IU setiap bulan selama 10 bulan (n = 93) atau plasebo (n = 99). Semua peserta adalah kekurangan (<50 nmol/l) vitamin D pada permulaan kajian. Hasil utama yang dikaji adalah serum 25(OH)D, profil serum lipid, tekanan darah dan HOMA-IR diukur pada permulaan kajian, bulan keenam dan bulan kedua belas. HRQOL dinilai dengan SF-36 pada permulaan kajian dan 12 bulan selepas itu. Sembilan puluh tiga dan 99 wanita dipilih secara rawak ke dalam kumpulan intervensi dan plasebo. Selepas 12 bulan, terdapat perbezaan yang signifikan di antara kumpulan rawatan dalam serum 25(OH)D (purata perbezaan: 49,54; 95% CI: 43,94-55,14 nmol/l) dan tahap PTH (purata perbezaan: -1,02; 95% CI : -1,67 ke -0,38 pmol/l) dalam kumpulan intervensi berbanding dengan kumpulan plasebo. Tiada kesan suplemen vitamin D dalam HOMA-IR, profil serum lipid dan tekanan darah (semua p> 0.05) antara dua kumpulan tersebut. Terdapat peningkatan yang kecil tetapi penting dalam HRQOL dalam komponen daya hidup (purata perbezaan: 5,041; 95% CI: 0,709-9,374) dan skor komponen mental (purata perbezaan: 2,951; 95% CI: 0,573-5,329) dalam

kumpulan interventi berbanding dengan kumpulan plasebo. Dos vitamin D yang tinggi dan kurang kerap adalah berkesan dalam memastikan tahap vitamin D yang mencukupi. Walau bagaimanapun, tidak ada peningkatan dalam faktor risiko kardiometabolik yang diukur pada wanita premenopausal. Walaubagaimanapun, suplemen vitamin D didapati boleh memperbaiki beberapa komponen HRQOL. Kajian longitudinal mungkin diperlukan untuk menentukan akibat vitamin D ke atas faktor-faktor risiko kardiometabolik dan HRQOL.

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ABBREVIATIONS

1,25(OH)D	1,25 hydroxyvitamin D
25(OH)D	25 hydroxyvitamin D
ANOVA	Analysis of Variance
ApoB	Apolipoprotein B
BDI	Beck Depression Index
BMI	Body Mass Index
BP	Blood Pressure
Ca	Calcium
CI	Confidence Interval
CPBA	Competitive Protein Binding Assay
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DM	Diabetes Mellitus
ECLIA	Electro-chemiluminescent Immunoassay
ELISA	Enzyme Linked Immunosorbent assay
FBS	Fasting Blood Sugar
HDL	High-density lipoprotein
HOMA-IR	Homeostasis Model Assessment – Insulin Resistant
HPLC	High-Pressure Liquid Chromatography
HRQOL	Health-Related Quality of Life
IOM	Institute of Medicine
IPAQ	International Physical Activity Questionnaire
IPAQ-M	International Physical Activity Questionnaire Malay
IQR	Inter-quartile range
IR	Insulin resistant
ITT	Intention-To-Treat
JOQOL	Japanese Osteoporosis Quality of Life
LC-MS/MS	Liquid Chromatography Tandem Mass Spectroscopy
LDL	Low-density lipoprotein
LME	Linear mixed effect
LOCF	Last observation carried forward

MCS	Mental Component Summary
MET-min	Metabolic equivalent minutes
NHMS	National Health Morbidity Survey
PABA	Para-aminobenzoic acid
PCS	Physical Component Summary
PTH	Parathyroid Hormone
QOL	Quality of Life
QUALEFFO	Quality of Life Questionnaire of The European Foundation for Osteoporosis
RCT	Randomized Controlled Trial
RDI	Recommended Daily Intake
RIA	Radioimmunoassay
RNI	Recommended Nutrient Intake
SBP	Systolic Blood Pressure
SD	Standard Deviation
SF-36	Rand 36-Item Health Survey
TG	Triglycerides
TNF	Tumour Necrosis Factor
UM	University of Malaya
UMMC	University Malaya Medical Centre
UVB	Ultraviolet B
VDR	Vitamin D Receptor
VLDL	Very low density lipoprotein
WC	Waist Circumference
WHO	World Health Organization

LIST OF APPENDICES

CHAPTER 1 : INTRODUCTION

1.1 Background

Vitamin D, also known as the “sunshine vitamin”, is a fat-soluble vitamin that is typically associated with bone health. Vitamin D deficiency is widely recognized as leading to rickets in children and osteomalacia and osteoporosis in adults (J. H. Lee, O'Keefe, Bell, Hensrud, & Holick, 2008). However, recent studies have suggested that in addition to its function in bone health, vitamin D may play an important role in the genesis of cardiovascular disease, metabolic syndrome, and certain cancer risk factors. Vitamin D deficiency may cause an increase in blood pressure by activating the renin-angiotensin-aldosterone system. Additionally, vitamin D deficiency increases the risk of arterial intimae thickness, vascular resistance, elevated triglycerides levels, insulin resistance, and inflammatory cytokines (Holick, 2011). These biologic actions may lead to the development of cardiometabolic risk factors, such as hypertension, diabetes mellitus, hyperlipidaemia, and inflammation. These factors increase the risk of cardiometabolic diseases. Moreover, vitamin D deficiency was found to be associated with depression, muscle pain and anxiety. Osteoporosis, osteomalacia, and hyperparathyroidism due to vitamin D deficiency may worsen bone health, eventually causing a reduction in health-related quality of life (HRQOL).

1.2 The rising epidemic of cardiometabolic diseases

Cardiometabolic diseases pose a significant threat to patients and remain a major global health problem. The World Health Organization (WHO) estimated that 17.3 million people died from cardiometabolic diseases in the year 2008, and this number is expected to continue to rise to 23.3 million people annually in 2030. Most cardiometabolic diseases can be prevented by addressing risk factors, such as tobacco

use, unhealthy diet and obesity, physical inactivity, high blood pressure (BP), high blood glucose, and elevated lipid levels.

In Malaysia, according to the 2011 National Health Morbidity Survey (NHMS), the prevalence of diabetes mellitus (DM) rose in just five years from 11.6% in 2006 to 15.2% in 2011:- during this time, hyperlipidaemia rose from 20.7% to 35.1% and hypertension rose from 32.2% to 32.7% (Institute of Public Health, 2011). This increase has had a serious socio-economic impact which requires solutions and strategies to prevent premature deaths from cardiometabolic disease through multi-sectoral mechanisms. The results from a nationwide survey on metabolic syndrome in Malaysia revealed that 42.5% of Malaysian adults suffered from metabolic syndrome. This value was higher in females (64.2%) (Mohamud et al., 2011) and was higher compared to other Asian countries (Deepa, Farooq, Datta, Deepa, & Mohan, 2007; Gu et al., 2005; Ko et al., 2006). This phenomenon may have a major impact on national healthcare and services costs, unless there is immediate intervention to reduce or prevent metabolic syndrome risk factors, such as hypertension, DM, and hypercholesterolaemia.

1.3 Epidemiology of vitamin D deficiency

1.3.1 Prevalence of vitamin D deficiency globally

Ultraviolet B (UVB) ray from the sun are a primary, inexpensive source of vitamin D compared to other natural sources of vitamin D, such as food, which contains very small amount of vitamin D. Nevertheless, the prevalence of vitamin D deficiency is still high across the globe. In the United States, the prevalence of vitamin D deficiency was between 25% and 57%, as reported in the Third National Health and Nutrition Examination Survey (NHANES III) (Kendrick, Targher, Smits, & Chonchol, 2009), whereas the prevalence of vitamin D deficiency was shown to be 28% in the Framingham Offspring Cohort study (T. J. Wang et al., 2008) and 47.8% in a study in

Amsterdam (Oosterwerff, Eekhoff, Heymans, Lips, & van Schoor, 2011). These levels remain high during the winter seasons, especially among the elderly and homebound geriatric patients (Gloth, Gundberg, Hollis, Haddad, & Tobin, 1995; Lips et al., 1988; Whitmore, 1996). Although vitamin D deficiency is typically common in people living in higher latitude countries, a high prevalence of vitamin D deficiency has also surprisingly been found in countries closer to the equator which are supposed to recieve sufficient sunlight throughout the year. Vitamin D deficiency has been reported at rates such as 61% in Iran (Hosseinpanah et al., 2011), 64.6% in Bangkok, Thailand (Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2011) and 66.3% in India (Mark F, 2009).

1.3.2 Prevalence of vitamin D deficiency in Malaysia

Malaysia is located at a latitude of 2° 30' north and is blessed with sufficient sunshine throughout the year necessary for cutaneous synthesis of vitamin D. Khor et al. (2011) reported that 35.3% of primary school children in Kuala Lumpur had vitamin D deficiency. Approximately 70% of Malay adults in Kuala Lumpur were found to suffer from vitamin D deficiency (Moy, 2011). In addition, both studies found an inverse association between vitamin D status and obesity. These findings have raised concerns, as the prevalence of obesity in Malaysia is on the rise (Kee et al., 2008). A study by Nurbazlin et al. (2013) showed that the UV index was the major factor influencing vitamin D status in Malaysian women. They found that 81.3% of urban women had low vitamin D status, as they spent less time exposed to sunlight compared to rural women (11.9%).

1.4 Vitamin D deficiency implications on health

1.4.1 Musculoskeletal diseases

Classically, vitamin D deficiency is known to cause musculoskeletal diseases such as rickets, osteomalacia, and osteoporosis, due to its functions in calcium absorption in the gut and the bone mineralization process. Vitamin D deficiency reduces the gut's ability to absorb sufficient calcium from the diet thus promoting the production of parathyroid hormone (PTH) and causing secondary hyperparathyroidism. This secondary hyperparathyroidism causes PTH to remove calcium from the bone to maintain the serum calcium level, and this process may increase the risk of osteoporosis and fracture (Holick, 2004; Laufey Steingrimsdottir, 2005).

1.4.2 Cardiometabolic risk factors

The term "cardiometabolic risk" refers to a situation in which the risks of developing atherosclerotic cardiovascular disease and diabetes mellitus are significantly enhanced due to insulin resistance (IR), high BP, high blood glucose and the presence of low high-density lipoprotein (HDL) and high triglyceride (TG) levels (Brunzell et al., 2008). Recent findings have recognized new roles of vitamin D in the human body, such as renin production in the kidneys, insulin production in the pancreas, the release of cytokines from lymphocytes, the production of cathelicidin in macrophages, and the growth and proliferation of both vascular smooth muscle cells and cardiomyocytes. The discoveries of these new functions may suggest non-musculoskeletal functions of vitamin D in humans, indicating that vitamin D may be associated with diseases, such as hypertension, diabetes mellitus, hyperlipidaemia, and cardiovascular diseases (Chowdhury, Boucher, & Hitman, 2009; Holick, 2011; Hosseinpanah et al., 2011; J. H. Lee et al., 2008).

1.4.3 Health-related quality of life (HRQOL)

As mentioned earlier, vitamin D plays an important role in reducing cardiometabolic disease and cancer risk factors, in addition to maintaining calcium and bone homeostasis. All these effects may lead to enhancements in physical function and quality of life (QOL). Muscle and bone pain is a common symptom of vitamin D deficiency as is depression. Vitamin D is a nuclear steroid hormone that is thought to be involved in brain health and function, as well as neuromuscular functions. In addition, vitamin D receptors in the cell's nucleus regulate the expression of target genes when bound to 1,25 hydroxyvitamin D (1,25(OH)D). These receptors are expressed in areas of the brain important for behavioural regulation (Kalueff & Tuohimaa, 2007).

Most studies on the effects of vitamin D levels on quality of life have largely focused on the elderly (S. Basaran, R. Guzel, I. Coskun-Benlidayi, & F. Guler-Uysal, 2007; Shahzad et al., 2009; M. D. Witham, L. J. Crighton, N. D. Gillespie, A. D. Struthers, & M. E. T. McMurdo, 2010b). All of these studies are related to the quality of life associated with skeletal diseases, such as osteoporosis and fracture and its effects on physical health and mental health. It is known that fractures cause pain and limited functional disability, leading to impaired QOL. In addition to physical health, vitamin D may have an effect on mental health. A study by Vieth et al (2004) concluded that an adequate intake of vitamin D was associated with improved mood and mental well-being. These findings were supported by another study in young adults in United States (Ganji, Milone, Cody, McCarty, & Wang, 2010). Nevertheless, current findings on the effects of vitamin D on physical and mental well-being remain inconsistent (S. Basaran et al., 2007; Ganji et al., 2010; Lansdowne & Provost, 1998; Shahzad et al., 2009; Vieth et al., 2004; Witham, Crighton, et al., 2010b). The question of whether vitamin D has a greater effect on QOL should be addressed using clinical trials to ascertain the relationship before any conclusions may be reached.

1.5 Prevention and treatment for vitamin D deficiency

The 2011 report on dietary reference from the Institute of Medicine suggested that an adequate intake of vitamin D for children and adults up to 70 years of age is between 400 IU/day and 800 IU/day (Ross et al., 2011). However, the Endocrine Practice Guidelines Committee recommended an intake of 400 to 600 IU/day, representing a lower level for vitamin D maintenance (Holick et al., 2011).

Vitamin D supplementation is the easiest approach to prevent vitamin D deficiency. Vitamin D supplements available over-the-counter can be in the form of either vitamin D2 or vitamin D3. In addition to supplementation, sufficient sunlight exposure is the least expensive way to prevent vitamin D deficiency. Sensible exposure to sunlight is effective in increasing serum 25 hydroxyvitamin D (25(OH)D) levels to a normal range (> 75 nmol/l or 30 ng/ml) . In addition, food fortification is another means of preventing vitamin D deficiency. Food fortification has the dual advantage of being able to deliver nutrients to large segments of the population, without requiring radical changes in food consumption patterns.

1.6 Rationale of the study

Vitamin D deficiency has recently been determined to be associated with cardiometabolic risk factors, along with its classical musculoskeletal functions, such as risk factors for rickets in children and increasing calcium absorption. This new role of vitamin D may be a new modifiable risk factor for cardiometabolic disease; however, more persuasive evidence is needed. Many observational studies have indicated an association between vitamin D and cardiometabolic risk (Hosseinpanah et al., 2011; Rejnmark, Vestergaard, Heickendorff, & Mosekilde, 2010; T. J. Wang et al., 2008). However, very limited studies have produced evidence of causal relationship between vitamin D and cardiometabolic risk. To date, only a few clinical trials have investigated

cardiometabolic risk as a primary outcome (Elamin et al., 2011; Grandi, Breitling, & Brenner; Pittas, Harris, Stark, & Dawson-Hughes, 2007; Thorand B. et al., 2011; von Hurst, Stonehouse, & Coad, 2010). The results of clinical trials, especially small clinical trials are often inconsistent in terms of the effects of vitamin D supplementation on cardiometabolic risk (Grandi et al.; Nagpal, Pande, & Bhartia, 2009; Pittas, Harris, et al., 2007; Rejnmark et al., 2010; von Hurst et al., 2010; Witham, Crighton, et al., 2010b; A. Zittermann et al., 2009). Therefore, there is a need to determine whether treating vitamin D deficiency using vitamin D supplementation may contribute to the prevention of cardiometabolic diseases.

More to the point, globally high rates of vitamin D deficiency and its continually increasing trends, as well as the appreciation of its association with cardiometabolic diseases, all suggest an ever-increasing awareness of the role of vitamin D in our health and wellbeing. Therefore, there is a need for conclusive evidence from randomized controlled trials (RCTs) to determine whether vitamin D supplementation can improve cardiometabolic risk factors.

1.7 Research questions

The research question for this study was "What is the effect of vitamin D supplementation on cardiometabolic risk factors and quality of life among vitamin D deficient premenopausal women?"

The above research question may be conceptualized by the 'PICO' concept described below:

P (population) : Vitamin D deficient premenopausal women

I (intervention) : Vitamin D supplementation

C (comparison) : Placebo (not taking vitamin D supplements)

O (outcomes) : Improvement in cardiometabolic risk factors and health-related quality of life

1.8 Hypothesis

i. Vitamin D supplementation in vitamin D deficient premenopausal women will result in an improvement in their vitamin D status.
ii. Vitamin D supplementation in vitamin D deficient premenopausal women will result in an improvement in cardiometabolic risk factors, including blood pressure (BP), homeostasis model assessment - insulin resistant (HOMA-IR), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL).
iii. Vitamin D supplementation will improve HRQOL in vitamin D deficient premenopausal women.

1.9 Conceptual framework

Figure 1.1 shows the concept for the trial framework. Factors known to cause vitamin D deficiency and ways in which vitamin D deficiency may affect cardiometabolic risk factors and health-related quality of life are outlined.

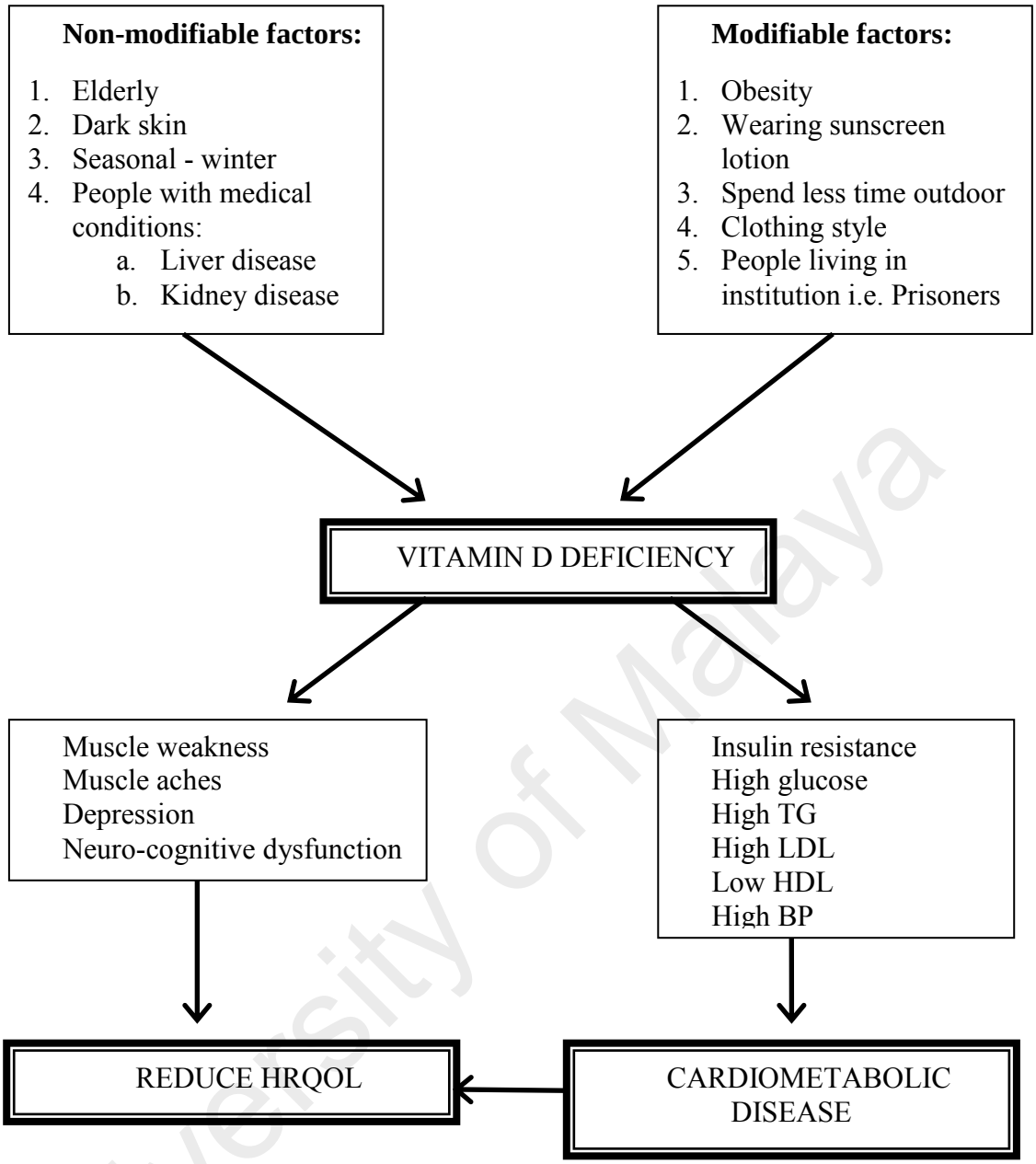

Figure 1.1: Conceptual framework of factors associated with vitamin D deficiency and its cardiometabolic and HRQOL consequences

Adapted from Holick (2011) and J. H. Lee et al. (2008)

1.10 Study objectives

1.10.1 General objectives

To determine whether vitamin D supplementation could improve cardiometabolic risk factors, such as BP, HOMA-IR, TG, HDL and LDL, as well as HRQOL in premenopausal women who were vitamin D deficient.

1.10.2 Specific objectives

i. To investigate whether vitamin D supplementation for 12 months could improve the vitamin D status of vitamin D deficient premenopausal women.

ii. To investigate whether vitamin D supplementation for 12 months could improves cardiometabolic risk factors, such as HOMA-IR, TG, LDL, HDL and BP, of premenopausal women with vitamin D deficiency.

iii. To investigate whether vitamin D supplementation for 12 months could improve the HRQOL of premenopausal women with vitamin D deficiency.

CHAPTER 2 : LITERATURE REVIEW

2.1 Introduction

This chapter reviews vitamin D sources and metabolism; the epidemiology, risk factors, health impacts, and treatment and prevention of vitamin D deficiency; and the questionnaires and statistical analysis used in this study.

2.2 Vitamin D

2.2.1 Vitamin D sources and metabolism

Natural vitamin D sources include food and UVB light. Skin exposure to UVB light from the sun is regarded as the main source of vitamin D as it provides more than 90% of the vitamin D requirement in humans. Flesh from fatty fish, such as salmon, tuna or mackerel and fish liver oils are among the best alternatives. Dried mushrooms enhanced with vitamin D2 from exposure to sunlight are also rich in vitamin D. In addition to these natural food sources, food fortified with vitamin D is also one of the best sources of vitamin D. In United States, milk is voluntarily fortified with 100 IU of vitamin D per cup, whereas in Canada, milk fortification with vitamin D (35 to 40 IU/100 ml) is required by law. Several food sources of vitamin D are listed in Table 2.1.

Table 2.1: Selected food sources of vitamin D

Sources	Vitamin D (IU per serving)
Natural sources:	
Cod liver oil, 1 tablespoon	1360
Sardines, canned, 3oz	300
Mushrooms, white, raw, 3 oz	100
Sun-dried shiitake mushrooms, 3 oz	1600
Egg yolk, raw	20
Salmon, fresh, 3 oz	447
Fortified food:	
Orange juice, 1 cup	137
Milk, non-fat, 1 cup	115

Source: National Nutrient Database for Standard Reference, USDA

Naturally vitamin D can be found in two forms. One is vitamin D2 or ergocalciferol which is the product of ultraviolet B (UVB) irradiation of ergosterol. Vitamin D2 can be found in plants and can be consumed as a supplement or in fortified food. The other form of vitamin D is vitamin D3 or cholecalciferol, which is usually found in oily fish, fortified foods and supplements. Vitamin D3 is also a product of UVB irradiation of 7-dehydrocholesterol in the human epidermis. Whether it is metabolized in the epidermis or ingested in the diet, vitamin D in the circulation is bound to vitamin D-binding protein, which transports it to the liver, where it is converted by vitamin D-25-hydroxylase to 25(OH)D, the major circulating form of vitamin D (Figure 2.1). However, 25(OH)D is biologically inert and is therefore converted to a biologically active form of vitamin D or 1,25(OH)D in the kidneys. In addition to the kidneys, other organs, such as the colon, prostate, and skin can also convert 25(OH)D to 1,25(OH)D.

The active forms of vitamin D (1,25(OH)D) then circulate in the body and binds to the vitamin D receptor (VDR), which is found in cells of the bone, pancreatic β-cells, parathyroid gland, brain, skin, prostate, skeletal muscles, intestines, kidneys, and adipose cells. VDR is also found in immune response cells, such as macrophages and activated T-cells, which are directly or indirectly involved in renin production in the kidney, insulin production in the pancreas, the release of cytokines from lymphocytes, the production of cathelicidin in macrophages, and the growth and proliferation of both vascular smooth muscle cells and cardiomyocytes (J. H. Lee et al., 2008). Vitamin D also travels to the intestine to enhance intestinal calcium absorption and to the skeleton to enhance the bone remodelling process by binding to the VDR in the intestines and osteoblasts. In addition, 1,25(OH)D feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone in the parathyroid glands.

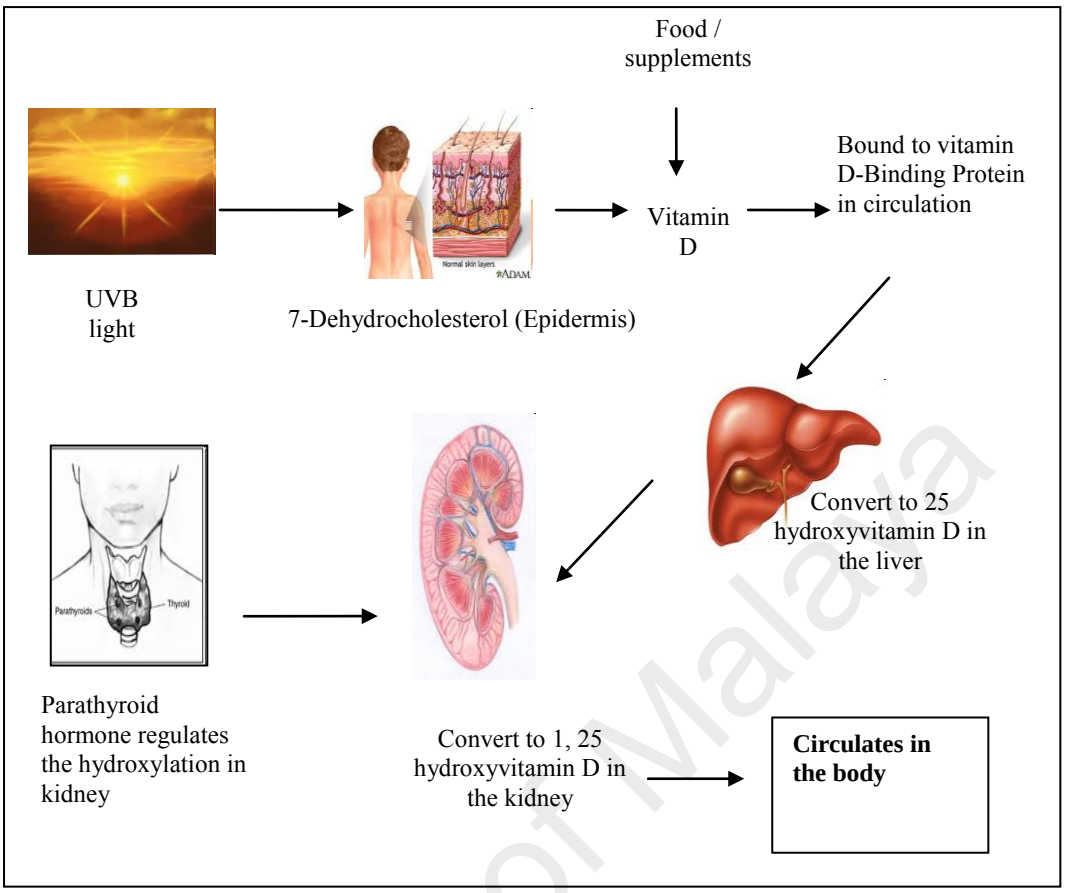

Figure 2.1: Metabolism and biologic actions of vitamin D in human body

Adapted from Holick (2011) and Reddy et al (2010)

2.2.2 Definition of vitamin D status

To date, there is little agreement or consensus among experts regarding the definition of vitamin D deficiency or as to what the normal range for serum 25(OH)D. The Institute of Medicine (IOM) Committee for 2011 Report on Dietary Reference Intake for Vitamin D (Ross et al., 2011) proposed a level of 50 nmol/l (20 ng/ml) of serum 25(OH)D as the optimum level of vitamin D because it fulfils the requirements of at least 97.5% of the population. However, this recommendation was primarily based on the intake and serum concentration of 25(OH)D needed to ensure skeletal health, not non-skeletal health.

It has been well established that elevated PTH concentration is associated with cardiometabolic diseases (A. Zittermann, 2006). Many studies have found an inverse relationship between serum PTH and serum 25(OH)D; furthermore, no significant changes in serum PTH were observed when the circulating serum 25(OH)D level was above 50 nmol/l before reaching a plateau at 75 nmol/l (Holick et al., 2005; Malabanan, Turner, & Holick, 1998; Thomas et al., 1998; A. Zittermann, 2006). Intestinal calcium absorption efficacy increases by 45% to 65% when the circulating serum 25(OH)D level is greater than 75 nmol/l (Heaney, Dowell, Hale, & Bendich, 2003). Therefore, based on these findings, Holick (2009) proposed a stricter definition for vitamin D deficiency. He defined vitamin D deficiency as a serum 25(OH)D level less than 50 nmol/l, and insufficiency as a 25(OH)D level of 52.5 to 72.5 nmol/l (21 to 29 ng/ml). A similar definition was used in the Endocrine Society Clinical Practice Guidelines (Holick et al., 2011). The Endocrine Society Guidelines recommendation was based on a medical model to prevent vitamin D deficiency and avoid other risks related to inadequate vitamin D status. Nevertheless, The Institute of Medicine (Ross et al., 2011) defined vitamin D deficiency as less than 30 nmol/l (12 ng/ml).

2.2.3 Recommended nutrient intake (RNI) of vitamin D

The Institute of Medicine (IOM) recommended a dietary intake of vitamin D of between 400 IU (10 μg) and 800 IU (20 μg), with an upper limit of 4000 IU (100 μg). However, many researchers considered this recommendation inadequate to increase vitamin D status to a level sufficent to prevent cardiovascular diseases (Heaney & Holick, 2011).

Generally, with every additional 100 IU of vitamin D per day, the serum 25(OH)D concentration increases by approximately 2.5 nmol/l (1 ng/ml) (Heaney, Davies, Chen, Holick, & Barger-Lux, 2003; Heaney, Recker, Grote, Horst, & Armas, 2011). Many studies have reported that at least 1000 to 4000 IU/day of vitamin D supplementation

was needed to sustain the circulating serum 25(OH)D level to above 75 nmol/l (30 ng/ml) (Nagpal et al., 2009; Tai, Need, Horowitz, & Chapman, 2008; von Hurst et al., 2010; Witham, Crighton, et al., 2010b; Witham, Dove, et al., 2010; A. Zittermann et al., 2009). Therefore, the RNI recommended by IOM is not sufficient to achieved the recommended serum 25(OH)D concentration. For individuals at risk of vitamin D deficiency, such as individuals who are overweight or obese, the elderly, and individuals with malabsorption syndrome, the recommended daily allowance (RDA) of 600 IU/day recommended by the IOM leads to barely appreciable changes (Heaney & Holick, 2011). For that reason, the Endocrine Society has developed Clinical Practice Guidelines for vitamin D intakes, which are recommended for patients at risk of vitamin D deficiency (Holick et al., 2011). They recommend that the daily requirement for adults be 1500 to 2000 IU with an upper limit up to 10,000 IU. This dosage might need to be increased to up to 3,000 IU per day for obese or elderly individuals, or populations with highly pigmented skin (Holick et al., 2011). The recommendations are based on a report that patients who received 10,000 IU/day of vitamin D for five months (Heaney, Davies, et al., 2003) and adults who received 50,000 IU of vitamin D every two weeks for six years (Pietras, Obayan, Cai, & Holick, 2009) showed no evidence of toxicity. Even the IOM report acknowledged that an intake up to 10,000 IU/day was likely safe because intakes lower than 10,000 IU/day have not been linked to hypercalcaemia or acute toxicity (Ross et al., 2011). Table 2.2 summarizes the vitamin D intakes recommended by the IOM and the Endocrine Practice Guidelines Committee according to age group.

Table 2.2: Vitamin D intakes recommended by the IOM and the Endocrine Practice Guidelines Committee

Age group	IOM recommendations		Endocrine Practice Guidelines Committee recommendations for patients at risk of vitamin D deficiency	
	RDA	UL	Daily requirements	UL
0 – 18 years	400 IU	1000 IU	400-1000 IU	2000 IU
19 – 50 years	600 IU	4000 IU	1500 – 2000 IU	10000 IU
> 50 years	600 - 800 IU	4000 IU	1500 – 2000 IU	10000 IU
Pregnant or lactating	600 IU	4000 IU	1500 – 2000 IU	10000 IU

RDA, recommended dietary allowances; UL, upper limit
Source: Holick et al. (2011)

Not only has the lower limit of the normal range of circulating serum 25(OH)D been questioned, the upper limit of the normal value of 125 nmol/l suggested by the IOM Committee (Ross et al., 2011) also appears inadequate. Individuals who are exposed to sunlight daily, such as lifeguards and farmers typically have reported serum 25(OH)D levels between 150 and 250 nmol/l and no reported vitamin D toxicity. Vitamin D toxicity is a clinical syndrome of hypervitaminosis D (greater than 375 nmol/l) with hypercalcaemia and hypercalciuria and it is considered as medical emergency. Patients with vitamin D toxicity have been reported to show signs of hypercalcaemia, such as nausea, dehydration, and constipation, as well as signs of hypercalciuria, such as polyuria and renal stones. However, serum 25(OH)D concentrations greater than 375 nmol/l alone, without signs of hypercalcemia and hypercalciuria, cannot be defined as vitamin D toxicity. Animal studies and human anecdotal reports of vitamin D intoxication indicated that the threshold for toxic symptoms can be as high as greater than 750 nmol/l (Jones, 2008). Vitamin D toxicity is extremely rare and usually occurs after the ingestion of large doses of vitamin D (more than 10,000 IU/day) over a prolonged period.

2.2.4 Methods of vitamin D assessment

As explained in section 2.2.1, UVB light is converted to serum 25(OH)D in the skin and then converted to 1,25(OH)D in the kidneys. Although serum 1,25(OH)D is the biologically active form of vitamin D, it is not commonly used to determine vitamin D status. The likely reason for this is that the half-life of circulating serum 1,25(OH)D is only four to six hours, compared to approximately two to three weeks for circulating serum 25(OH)D (Holick, 1989).

Under vitamin D deficient conditions, calcium sensors in the parathyroid glands receive a signal due to a decrease in intestinal calcium absorption as a result of vitamin D deficiency. The parathyroid glands then produce PTH, which then increases the renal production of 1,25(OH)D. Therefore, under vitamin D deficiency condition, the serum 1,25(OH)D level might be normal or higher compared to the serum 25(OH)D level, which remains low. Serum 25(OH)D is the major circulating metabolite of vitamin D and reflects vitamin D input from skin synthesis and dietary intake; therefore, it is commonly used as the standard clinical measure of vitamin D status.

Demand for 25(OH)D and 1,25(OH)D assays has substantially increased worldwide. There are many commercially available serum 25(OH)D assays used to determine vitamin D status. These assays include competitive protein binding assays (CPBA), radioimmunoassays (RIA), high-pressure liquid chromatography (HPLC), enzyme-linked immunosorbent assays (ELISA), liquid chromatography tandem mass spectroscopy (LC-MS/MS) and electrochemiluminescence immunoassays (ECLIA). Because there is no standard laboratory assay to measure circulating serum 25(OH)D, both clinical and research settings make use of a variety of assays to measure vitamin D status, which may yield some discrepancies in the results.Thesediscrepancies have raised concerns about the comparability and accuracy of the different assays. Therefore, it is difficult to compare the results from studies investigating the prevalence or clinical

consequences of vitamin D deficiency. A study by Snellman et al. (2010) comparing commercially available assays found that due to substantial inter-assay differences in performance, it was difficult to define an optimal serum 25(OH)D level.

The following are the various assays used to assess 25(OHD) levels:

i. Competitive protein binding assay (CPBA)

In addition to measuring serum 25(OH)D and 1,25(OH)D, most assays are usually able to measure both circulating serum 25 dihydroxyvitamin D2 (25(OH)D2) and 25 dihydroxyvitamin D3 (25(OH)D3). The first assays used to measure 25(OH)D was the CBPA format with vitamin D binding protein as the binder. The advantage of using this assays is that it recognizes both 25(OH)D2 and 25(OH)D3 equally. However, its major disadvantage is that it measures other vitamin D metabolites in addition to 25(OH)D, including 24,25-dihydroxyvitamin D and 25,26-dihydroxyvitamin D. Studies that used CBPA assays included Aljabri et al. (2010), Breslavsky et al. (2013) and Pittas et al. (2007).

ii. Radioimmunoassay (RIA)

RIA was developed to measure 25(OH)D and is able to recognize 25(OH)D2 and 25(OH)D3 equally well. However, similar to CBPA assays, it also recognizes other polar metabolites to the same extent and typically overestimates 25(OH)D levels by approximately 10 to 15%.

RIA is available from two companies, namely DiaSorin (DiaSorin, Stillwater, MN), as approved by the Food and Drug Administration for clinical use in the United States, and IDS (Immunodiagnostic Systems Inc, Fountain Hills, AZ). IDS RIA uses a two-step extraction procedure,whereasthe DiaSorin RIA procedure hasonly one step. A study by Hollis (2000) found that IDS RIA underestimated 25(OH)D on average by ≥ 30% compared to Diasorin RIA and HPLC method.

Studies that used RIA assays include Jorde et al. (2010), Pfeifer et al. (2001), Sakalli et al. (2012), Schreuder et al. (2012), Tai et al. (2008), (2013), von Hurst et al. (2010), M.D. Witham et al. (2010a), Witham et al. (2010) and A. Zittermann et al. (2009).

iii. High-pressure liquid chromatography (HPLC)

To avoid the overestimation of circulating serum 25(OH)D levels due to polar metabolites, an HPLC assay can be used because it is able to separate serum 25(OH)D from more polar metabolites that interfered with assays. HPLC is considered the standard assay but is very cumbersome because it requires a large sample volume and well-trained technicians; thus, it is not preferred for use in clinical samples or large studies. The procedure for this assay includes serum lipid extraction followed by preparative chromatography. Then, the 25(OH)D fraction must be applied to HPLC, and the ultraviolet absorption of 25(OH)D is used to measure the 25(OH)D concentration. Due to its cumbersome procedure, this method is used in few clinical studies. Only a few observational studies have employed the HPLC assay (Sibel Basaran, Rengin Guzel, Ilke Coskun-Benlidayi, & Fusun Guler-Uysal, 2007; Ecemis & Atmaca, 2013; W. Huang, Shah, Long, Crankshaw, & Tangpricha, 2013; Salekzamani et al., 2011).

iv. Enzyme-linked immunosorbent assay (ELISA)

ELISA is another commercially available assay to measure circulating serum 25(OH)D. Similar to RIA, ELISA assays are available from two companies, Diasorin and ImmunoDiagnostic System (IDS). The antibody used in ELISA by IDS has been reported to have 75% cross-reactivity with 25(OH)D2, which is likely the same as the RIA by IDS (Zerwekh, 2008). However, IDS Diasorin has only 23% cross-reactivity with 25(OH)D2. Although there are differences in cross-reactivity and procedure for the same assays developed by different companies, it is difficult to identify which studies have used which assays because most studies do not mention the companies that developed the assays that they used. Although this assay is not considered the gold

standard assay for measuring serum 25(OH)D, many prospective clinical studies (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Al-Daghri, Alkharfy, Al-Saleh, et al., 2012; Asemi, Hashemi, Karamali, Samimi, & Esmaillzadeh, 2013; Salehpour et al., 2012; Yiu et al., 2013) and observational studies (Anand et al., 2011; Barbara Thorand, 2011; Botella-Carretero et al., 2007) have used ELISA as their method of choice.

v. Electrochemiluminescent immunoassay (ECLIA)

ECLIA is another method for measuring circulating serum 25(OH)D. Two types of assay use this method: (1) the LIAISON 25(OH)D vitamin D TOTAL Assay and (2) the Roche vitamin D3 assay. The LIAISON vitamin D TOTAL Assay is a direct competitive chemiluminescence immunoassay intended for used on the DiaSorin LIAISON automated analyzer whereas the Roche vitamin D3 assay is a direct competitive electrochemiluminescence immunoassay intended for use on a Roche automated immunoassay analyzer (Cobas E-411). This method is preferred by many researchers because LIAISON analyzers or Cobas E-411 analyzers are available in many laboratories (Gagnon et al., 2012; Gagnon et al., 2011; Jorde et al., 2010; K. C. Maki et al., 2011; Moy, 2011; Moy & Bulgiba, 2011; Vacek et al., 2012). A study comparing these two assays with RIA assay to measure circulating serum 25(OH)D concluded that the LIAISON assay exhibited better correlation and agreement with DiaSorin RIA assays for serum 25(OH)D determination than the Roche ECLIA assays (Wagner, Hanwell, & Vieth, 2009).

vi. Liquid chromatography tandem mass spectroscopy (LC-MS/MS)

The last method, which is the most advanced assay to date, is the LC-MS/MS. This assay is applied for the direct measurement of 25(OH)D in human serum. It's able to quantify both 25(OH)D2 and 25(OH)D3 separately and accurately. This method however, is best suited to high-volume reference laboratories as it requires derivatization or deuterated internal standards. Because LC-MS/MS is a complicated

procedure, only a few studies have used this method (Dean et al., 2011; 2011; Sollid et al., 2014).

A summary of vitamin D assessment assays are presented in Table 2.3. To choose a method, a researcher may need to consider many factors. Without the appropriate equipment and expertise, using some of the methods in an in-house laboratory instead of commercial laboratories may be inefficient and difficult. More accurate methods, such as HPLC and LC-MS/MS may need to be used in commercial laboratories because of their cumbersome procedures; however, they may be preferred if the research budget allows. Nevertheless, a study by Lips et al (1999) found that the mean serum 25(OH)D value differred by only 38% between the laboratories (assays) with the highest and lowest values, and the ranking order of individual samples was almost the same between the assays. Therefore, the choice of method depends on the availability of the appropriate equipment and the expertise of the laboratory rather than which assays yield the most accurate results. Furthermore, unless a careful cross-calibration has been performed on different laboratories or assays, the results cannot be assumed to be comparable.

Most current assays can use either serum or plasma, which can be stored at -20°C until analysis. In addition, samples are not affected by exposure to UV light, heat, or repeated freezing and thawing cycles of the serum (Antoniucci, Black, & Sellmeyer, 2005; Lissner, Mason, & Posen, 1981).

Table 2.3: Summary of serum 25(OH)D assessment assays

Assays	Sample type / volume	Comments
CBPA	Serum or plasma / 50µL	Uses DBP in EIA format 100% cross-reactivity with 25(OH)D
RIA	Serum or plasma / 50µL	Calibrators and controls in serum matrix; no yield determination required
HPLC	Serum / 500µL	Laboratory must have HPLC unit with silica column 25(OH)D2 and 25(OH)D3 separated with different column
ECLIA	Serum or plasma / 25µL	Fully automated on Liaison instrument 100% cross-reactivity with 25(OH)D2
LC-MS/MS	Serum / 25µL	Accurately quantifies both 25(OH)D2 and 25(OH)D3. Requires derivatization or deuterated internal standards

Adapted from Zerwekh (2008)

2.2.5 Epidemiology of vitamin D deficiency

2.2.5.1 Global prevalence of vitamin D deficiency

The global prevalence of vitamin D deficiency is high, especially in high-latitude countries, becausea key factor influencing the amount of UVB radiation that reaches the Earth's surface is the solar zenith angle. The solar zenith angle is the angle between the local vertical (zenith) and a line from the observer to the sun. A smaller solar zenith angle results in more intense ultraviolet radiation; thus, more vitamin D can be synthesized in the skin. At latitudes higher than approximately 35° north, vitamin D synthesis is impossible during the winter months and only reaches its maximum at mid-day in the summer (Tsiaras & Weinstock, 2011).

In higher-latitude countries such as the United States, the prevalence of vitamin D deficiency is between 25% and 57% (Kendrick et al., 2009). However, the prevalence of vitamin D deficiency was found to be 28% (less than 37.5 nmol/l) in the Framingham Offspring Cohort study (T. J. Wang et al., 2008) and 70.3% (less than 75 nmol/l) in a study by Vacek et al. (2012). In Spain, at a latitude of 40.4° north, the prevalence of vitamin D deficiency in morbidly obese individuals is only 50.7% (Botella-Carretero et

al., 2007). Ten degrees further north in Amsterdam (latitudes of 52.4° north), the prevalence of vitamin D deficiency is 36.9% (less than 50 nmol/l) (Oosterwerff et al., 2011). This prevalence remains high during the winter seasons, especially in elderly and homebound geriatric patients (Gloth et al., 1995; Lips et al., 1988; Whitmore, 1996).

Although vitamin D deficiency is common among people living in higher-latitude countries, a higher prevalence of vitamin D deficiency is also surprisingly found in countries closer to the equator,which typically receive sufficient sunlight throughout the year. Some rates of vitamin D deficiency reported by such countries include 61% (less than 37.5 nmol/l) in Iran (latitude of 32°north) (Hosseinpanah et al., 2011), 64.6% (less than 75 nmol/l) in Bangkok, Thailand (latitudes of 13.7° north) (Chailurkit et al., 2011) and 76.3% (< 50 nmol/l) in reproductive women in Southern India (latitudes of 13.4° north) (Harinarayan et al., 2011).

Figure 2.2 presents a global representation of vitamin D status in healthy adult populations (Wahl et al., 2012). Vitamin D status is approximately split between 24 to 49 nmol/L and 50 to 74 nmol/L globally, which is still below the sufficiency levels especially in countries at higher latitudes. However, there is an information gap on vitamin D status in some regions, especially in Africa and South America. It is important to fill this gap so that the appropriate public health measures can be implemented to improve the vitamin D status thus preventing the health implications of vitamin D deficiency.

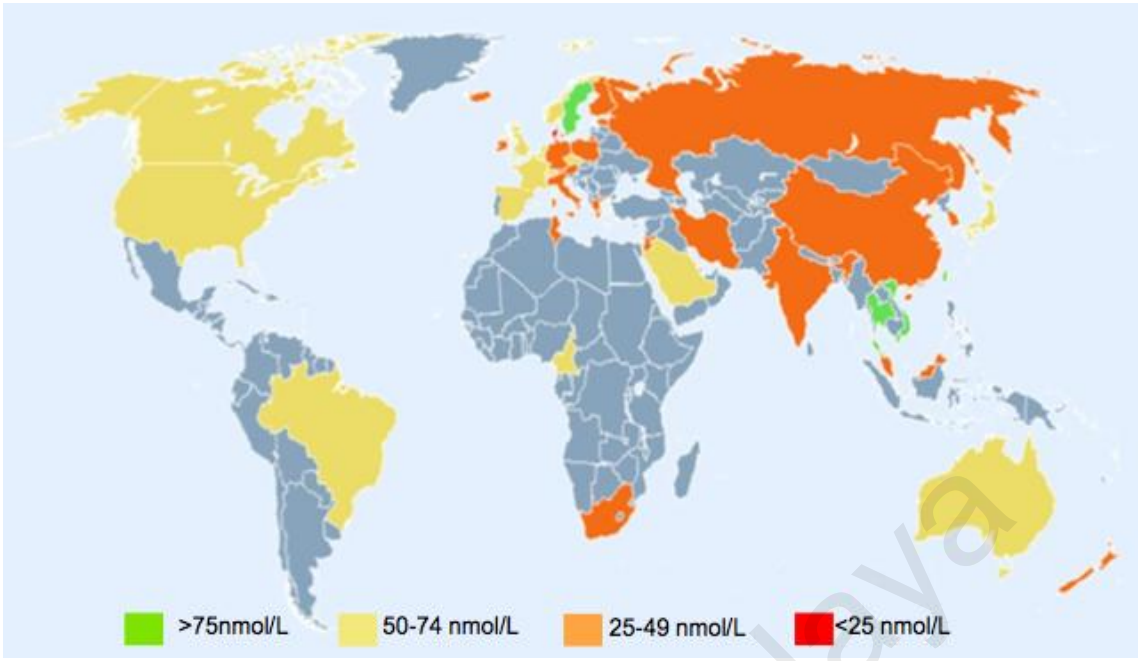

Figure 2.2: A global representation of vitamin D status in healthy populations

Source: Wahl et al. (2012)

2.2.5.2 Prevalence of vitamin D deficiency in Malaysia

Malaysia is located at latitude 3° north and is therefore blessed with sufficient sunshine throughout the year necessary for the cutaneous synthesis of vitamin D. Although Malaysia is located near the equator, many researchers havefound that sizeable proportions of Malaysians have inadequate vitamin D status (Green et al., 2008; Khor et al., 2011; Moy, 2011; Nurbazlin et al., 2013; Rahman, Chee, Yassin, & Chan, 2004). A study on vitamin D status in postmenopausal Malay women reported that 73.3% serum vitamin D concentration less than 50 nmol/l (Rahman et al., 2004), and Green et al. (2008) reported that over 60% of women of child-bearing age suffered from vitamin D deficiency (less than 50 nmol/l). Moy (2011) also reported that a high prevalence of urban females in Kuala Lumpur had vitamin D deficiency (86.9%). This study showed that females were 2.9 fold more likely to have vitamin D insufficiency than males. Similar findings were reported by Nurbazlin et al. (2013) who found that 81.3% of urban women had vitamin D levels less than 50 nmol/L compared to rural

women (11.6%). Unexpectedly, a large proportion of primary school children (72.4%) were also reported to be vitamin D deficient (Khor et al., 2011).

2.2.6 Risk factors for vitamin D deficiency

Factors influencing vitamin D status can be classified as modifiable or non-modifiable (Table 2.4). Modifiable risk factors can be targeted for primary prevention purposes whereas non-modifiable risk factors can be targeted for secondary prevention purposes, such as regular screening and supplementation.

Table 2.4: Modifiable and non-modifiable risk factors for vitamin D deficiency

Modifiable risk factors	Non-modifiable risk factors
Obesity	Age (elderly)
Sunlight avoidance:	People with medical condition:
a. Wearing sunblock lotion	a. Liver disease
b. Seldom spend time outdoors	b. Kidney disease
c. Clothing styles	c. Malabsorption syndrome
Physical inactivity	Living in high latitude
Low vitamin D dietary intake	Highly-pigmented skin, e.g. Indian, African

2.2.6.1 Modifiable risk-factors

a) Obesity

Obese person are at higher risk of vitamin D deficiency because excess adipose tissue leads to a decrease in the bioavailability of vitamin D, which is restored in the fat tissues. A study by Wortsman et al. (2000) found that obese individuals (body mass index [BMI] greater than 30 kg/m^2) had serum vitamin D levels 57% lower than in age-matched, normal-weight controls (BMI = 23 to 25 kg/m^2) after UVB irradiation. They also found that BMI was inversely correlated with peak serum vitamin D concentrations after supplementation with 50,000 IU of vitamin D. These findings are similar to many studies that have shown an inverse association between vitamin D status and obesity (K. Brock et al., 2010; Kim et al., 2010; Kevin C. Maki et al., 2009; Moy & Bulgiba, 2011; Nagpal et al., 2009; Tzotzas et al., 2010). Two studies found that improvement in

vitamin D status in overweight and obese subjects during a weight-loss intervention programme led to improvements inseveral cardiovascular disease (CVD) risk markers (Major, Alarie, Dore, Phouttama, & Tremblay, 2007; A. Zittermann et al., 2009). Therefore, early vitamin D screening in obese individuals is important, as individuals with BMI greater than 30 kg/m^2 require at least two to five fold increase in their vitamin D requirement to prevent the consequences of vitamin D deficiency.

b) Sunlight avoidance

Sunlight avoidance is also considered a modifiable risk factor for vitamin D deficiency because any interference in UVB light reaching the skin can be a factor causing vitamin D deficiency. Sunlight avoidance includes sunblock usage, certain clothing styles, and spending less time outdoors.

The reasons for sunlight avoidance might include concerns about health effects from excessive sunlight exposure, such as skin cancer. Public health campaigns regarding the increased risk of skin cancer due to excessive sun exposure in many countries, such as Australia, has caused an increase in the prevalence of vitamin D deficiency. A study found that 85% of the Australian dermatologists who were actively involved in a public health campaign promoting sunlight avoidance to reduce the risk of skin cancer were vitamin D deficient (less than 50 nmol/l) (Czarnecki, Meehan, & Bruce, 2009). Another reason for sunlight avoidance could be cosmetic. In a qualitative study conducted in Australia, most immigrant women (Chinese and Korean) reported that they preferred fair skin because they believed that fair skin was more beautiful than tanned skin; this was their reason for active avoidance of sunlight exposure (Jang et al., 2013).

i. Wearing sunblock lotion

Sunblock lotion interferes with UVB-7-dehydrocholesterol interactions by absorbing, reflecting or scattering incident ultraviolet radiation. The photo conversion of 7-dehydrocholesterol to pre-vitamin D in the skin is disrupted by the application of 5% para-aminobenzoic acid (PABA) (Tsiaras & Weinstock, 2011). Application of sunblock lotion before going outdoors increases the risk of vitamin D deficiency, as the common sun protection factor of 30 (SPF-30) lotion reduces vitamin D photosynthesis in the skin by more than 95%.

ii. Spending less time outdoors

Working indoors prevents the skin from receiving sufficient sunlight for vitamin D photosynthesis. Moy (2011) studied a population working in a public university, of whom more than 50% worked indoors more than eight hours per day. They found that more than 67.5% of the subjects were vitamin D deficient despite living in a country with abundant sunlight.

iii. Clothing style

Clothing style plays a significant role in reducing the UVB light that reaches the epidermis. Some people cover more than 95% of the skin due to cultural or religious reasons. Thus, their risk of becoming vitamin D deficient is higher compared to people who expose at least 50% of their skin. Studies have reported that Muslim women wearing a veil or hijab covering their body and head but not the hands or face, had a higher risk of vitamin D deficiency (Aljabri et al., 2010; Moy, 2011; Nazarian, St Peter, Boston, Jones, & Mariash, 2011; Neyestani et al., 2011). In addition to clothing covering almost 90% of the body surface, types of textiles or qualities of fabrics used as clothing influence the amount of UVB light that reaches the epidermis for vitamin D synthesis. Fabrics such as wool, silk, nylon, and polyester are more effective at preventing ultraviolet radiation from reaching the skin than lightweight cotton and linen

(Davis, Capjack, Kerr, & Fedosejevs, 1997). In addition, white fabrics (47.7%) are less effective at reducing UVB exposure compared to black coloured fabrics (98.6%) (Matsuoka et al., 1992).

c) Physical inactivity

Many studies have found an association between physical activity and vitamin D status (Anand et al., 2011; K. Brock, Cant, Clemson, Mason, & Fraser, 2007; K. Brock et al., 2010; De Rui et al., 2014; Foo et al., 2009; van Dam et al., 2007; Van den Heuvel, Van Schoor, De Jongh, Visser, & Lips, 2013). The participants who engaged in vigorous activity outdoors tend to have higher vitamin D status compared to those who did not (K. Brock et al., 2007). However, this finding may indicate that the participants engaging in outdoor physical activity, which causes more vitamin D synthesis in the skin, are more exposed to sunlight than those who spend more time indoors. The participants with low serum 25(OH)D had significantly greater muscle weakness, which may have limited their capacity for exercise outdoors (Heike A. Bischoff-Ferrari, 2007; Boland, 1986; Ceglia, 2008). Nevertheless, a study by De Rui et al. (2014) produced similar findings: subjects who participated in regular cycling and gardening had higher serum 25(OH)D levels irrespective of age, BMI, or season of the year. The same study found no associations between vitamin D status and other outdoor activities, such as walking and jogging. However, no studies have investigated the role of physical activity and vitamin D bioavailability and metabolism to identify the biological reasons for the association between vigorous physical activity and vitamin D status.

d) Low vitamin D dietary intake

Dietary vitamin D has been consistently reported as important for vitamin D status although the vitamin D content in food is low (K. Brock et al., 2007; K. Brock et al., 2010; K. E. Brock et al., 2013; van Dam et al., 2007). During the winter, people depend on a high intake of fatty fish and dairy products or vitamin D-fortified food as a vitamin

D source. In a study in the Netherlands, the consumption of vitamin D-fortified margarine products, fatty fish, and red meat was found to be associated with better vitamin D status (van Dam et al., 2007). K. Brock et al. (2010) also found that milk intake was a significant source of vitamin D in the United States. This result could be due to the mandatory vitamin D fortification of dairy products in the United States. However, in Australia, where dairy products are not fortified with vitamin D, increased dairy product consumption was still found to be associated with higher vitamin D status (K. Brock et al., 2007; K. E. Brock et al., 2013). A possible explanation for this finding may be that increased dairy intake leads to increased calcium intake, which is known to reduce the rate of degradation of 25(OH)D (Clements, Johnson, & Fraser, 1987) thus leading to improvement in vitamin D status. A study by Istiany et al. (2012) found that serum 25(OH)D could be increased through nutrition education and through vitamin D supplementation and sun exposure.

2.2.6.2 Non-modifiable risk factors

a) Age (elderly)

Ageing is known to reduce the body's ability to produce vitamin D, leading to vitamin D deficiency (Hsia et al., 2007; Oosterwerff et al., 2011; Rejnmark et al., 2010; Witham, Crighton, et al., 2010b). The elderly tends to spend more time indoors and are usually physically inactive, causing them to be prone to have vitamin D deficiency. Ageing can reduce the skin's ability to allow UVB light to penetrate the epidermis for vitamin D synthesis (Holick, Matsuoka, & Wortsman, 1989). A study by MacLaughlin and Holick (1985) found that the amount of pre-vitamin D3 produced in the skin of subjects aged more than 70 years was two-fold lower compared to individuals aged less than 18 years.

b) People with medical conditions

Other factors that can cause vitamin D deficiency are medical conditions, such as liver or kidney failure, granulomatous disorders, and malabsorption syndromes. Because the metabolism of vitamin D into its active form occurs in organs such as liver and kidney, people with any diseases that affect these organs are at risk of vitamin D deficiency. Granulomatous disorders, such as sarcoidosis and tuberculosis cause hypercalcaemia secondary to elevate to 1,25(OH)D. The increase in serum 1,25(OH)D is due to the extra-renal conversion of 25(OH)D to 1,25(OH)D in activated macrophages, thus reducing the circulating serum 25(OH)D in the body and causing vitamin D deficiency. Patient's with malabsorption syndrome, such as Crohn's disease or cystic fibrosis, and patients who have undergone gastric bypass surgery are also at risk of vitamin D deficiency. These patients are unable to absorb vitamin D efficiently and often require much higher doses of vitamin D supplements to meet their vitamin D requirement (Holick, 2006).

c) Living at high latitudes

UVB light from the sun is needed to metabolize 7-dehydrocholesterol in the epidermis into vitamin D. Because vitamin D synthesis requires sunlight for metabolism in the skin, the amount of UVB light that reaches the Earth must be sufficient to enable this process. The amount of UVB light that reaches the Earth depends on the zenith angle, or the angle of the sunlight from the sun to the Earth. For maximum UVB light to reach the Earth, the zenith angle must be upright. However, during the winter, at higher latitudes, or in the early morning and late afternoon, the zenith angle is usually oblique. At this angle, sunlight must pass through the ozone layer. The ozone layer efficiently absorbs UVB rays, causing very few UVB photons to reach the Earth. Therefore, people living at northern latitudes are more prone to vitamin D deficiency, especially during the winter (Botella-Carretero et al., 2007; K. Brock et al., 2010; Hsia et al., 2007; Kim

et al., 2010; Kevin C. Maki et al., 2009; Thorand B. et al., 2011; von Hurst et al., 2010; T. J. Wang et al., 2008; A. Zittermann et al., 2009). Although this fact has been demonstrated by many studies, it cannot be denied that people living in countries closer to the equator are also prone to vitamin D deficiency. Therefore, living in higher latitudes countries is not a major cause of vitamin D deficiency.

d) Highly pigmented skin

Melanin is the skin pigment that acts as a natural sunblock, protecting the skin from UVB ray penetration. Dark-skinned individuals, such as Indians, have high skin pigmentation, which provides the equivalent of at least an SPF of eight (SPF-8) and reduces the efficacy of vitamin D3 production in their skin by 50% to 90%. Thus, people with dark skin pigmentation are also at risk of vitamin D deficiency. A study by Clemens et al. (1982) found that African-American adults require at least five- to 10-fold longer exposure to sunlight than white adults to increase their serum vitamin D levels 10- to 20-fold. Based on the Fitzpatrick skin type classification, people with skin type 4 (brown) or 5 (dark brown) have a greater capacity to produce melanin, which protects them from UVB light penetrating the skin (Fitzpatrick, 1988). Most Malaysians are skin type 4 or type 5, except for Malaysians of Chinese origin, who have skin type 2.

2.2.7 Vitamin D implications for musculoskeletal condition

Vitamin D deficiency in children causes rickets (Holick, 2004). This is a condition of defective mineralization or calcification of bones, causing bone deformation. Rickets occurs when children are unable to absorb sufficient calcium due to vitamin D deficiency, which results in an increase in PTH, causing the removal of calcium from the bone to maintain the blood calcium levels essential for neuromuscular and other metabolic activities. Consequently, children with this condition begin to lose 0.5% of

their bone mass per year, which will increase their risk of osteoporosis and fracture if this condition continues for over a period of 10 to 20 years (Holick, 2006).

In adults, a similar mineralization defect of the bones can occur, but they tend not to develop overt bone deformities because adults have sufficient minerals in the bone. Instead, the defective bone mineralization prevents collagen from being properly mineralized, causing osteomalacia.

In menopausal women, vitamin D deficiency exacerbates bone mass loss, which occurs due to the loss of oestrogen stimulation in the bone. This phenomenon increases their risk of developing osteoporosis, putting them at higher risk of fracture.

2.2.8 Vitamin D implications for cardiometabolic risk factors

2.2.8.1 Insulin resistance

Insulin resistance is defined as the diminished ability of cells to respond to insulin,causing reduced glucose transport from the blood stream into cells. Insulin resistance plays a major role in the pathogenesis of type 2 diabetes mellitus and in cardiovascular risk, and it is recognized as one of the components of metabolic syndrome. The potential mechanisms by which an improvement in vitamin D status may have some effect on insulin resistance include modulating the generation and effects of cytokines, which are important for enhancing insulin sensitivity and promoting β-cell survival (Pittas, Lau, Hu, & Dawson-Hughes, 2007). In addition, vitamin D insufficiency induces elevations in the PTH level, which could adversely affect glucose metabolism by suppressing insulin signalling by adipocytes in hyperparathyroidism, thus inducing insulin resistance.

Surrogate markers used to gauge insulin resistance vary from invasive and expensive procedures to simple tests involving a single fasting blood sample (Singh & Saxena, 2010). Insulin resistance can be estimated using fasting blood samples (serum insulin

and blood glucose) to calculate simple indices, such as HOMA-IR or the Quantitative Insulin Sensitivity Check Index (QUICKI), or by performing the gold standard hyperinsulinaemic-euglycaemic clamp test. However, the gold standard clamp test is very expensive and not commonly used in epidemiological studies. The homeostasis model assessment (HOMA) was first introduced by Matthews et al. (1985). HOMA is a simple method used to quantify insulin resistance and beta-cell function from fasting glucose and insulin concentration using a simple mathematical linear equation; HOMA-IR = (fasting glucose x fasting insulin) / 22.5. Findings by Bonora et al. (2000) demonstrated that HOMA-IR could be reliably used in epidemiological studies because it closely mirrors the gold standard (glucose clamp technique) in the assessment of insulin sensitivity to predict type 2 DM. Many studies evaluating the benefits of vitamin D for DM have used HOMA-IR to measure insulin resistance (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Asemi et al., 2014; Breslavsky et al., 2013; Pittas, Harris, et al., 2007; Ryu et al., 2013; Salekzamani et al., 2011; Sollid et al., 2014; Strobel et al., 2014; Talaei et al., 2013; Wood et al., 2012).

2.2.8.2 High blood glucose

There are few potential mechanisms that either directly or indirectly support the association between vitamin D and high blood glucose (Pittas, Lau, et al., 2007). One of the direct mechanism is mediated by the binding of 1,25 (OH)D, the active form of vitamin D to the pancreatic β-cell VDR, which later induces insulin secretion. In addition, vitamin D helps to stimulate the expression of insulin receptors, enhancing insulin sensitivity and promoting glucose transport. Vitamin D regulates extracellular calcium which improves calcium flux in pancreatic β-cells and subsequently induces insulin secretion (Manna & Jain, 2012; Zhou et al., 2008).

Many observational and clinical trials have strongly supported the role of vitamin D deficiency in the pathogenesis of type 2 diabetes mellitus (Afzal, Bojesen, &

Nordestgaard, 2013; Ahmadieh, Azar, Lakkis, & Arabi, 2013; Al-Daghri et al., 2014; Asemi et al., 2013; Blanton et al., 2011; Chacko et al., 2011; Chailurkit, Aekplakorn, & Ongphiphadhanakul, 2012). Observational studies such as the NHANES III have found a very strong inverse association between serum 25(OH)D and the prevalence of diabetes mellitus (Fraser, Williams, & Lawlor, 2010). A case cohort study by Thorand B. et al. (2011) found similar results, concluding that higher serum vitamin D level were associated with a lower risk of type 2 diabetes mellitus with a hazard ratio of 0.52. Both studies had large sample sizes.

However, studies using small numbers of samples have produced inconsistent findings. A case control study from Iran found that lower serum vitamin D status was associated with higher glucose levels in patients with metabolic syndrome (Neyestani et al., 2011) and similar findings have been reported elsewhere (Aljabri et al., 2010; Gagnon et al., 2011; Nagpal et al., 2009; Nazarian et al., 2011; Pittas et al., 2006; Pittas, Harris, et al., 2007). On the other hand, some studies have found no association between vitamin D status and diabetes or glucose level (Moy & Bulgiba, 2011; Tai et al., 2008; von Hurst et al., 2010; Witham, Dove, et al., 2010; A. Zittermann et al., 2009).

2.2.8.3 Abnormal lipid profile

The mechanism by which vitamin D deficiency causes abnormal lipid profiles is still unclear. According to one theory, vitamin D exerts an anti-hyperlipidaemia effect by reducing hepatic TG formation and secretion via an increase in intestinal calcium absorption, as suggested by Zitterman et al (2009). In addition to reducing hepatic TG formation, this mechanism would increase the intracellular calcium in hepatocytes, thus stimulating the microsomal triacylglycerol transfer proteins (MTPs) involved in the formation of very-low-density lipid (VLDL) and TG (Van der Meer et al., 1990; Vaskonen, 2003).

Vitamin D influences serum lipid levels indirectly through its relation with the serum PTH level. PTH is reported to reduce lipolysis; therefore, vitamin D supplementation indirectly increases lipolysis by suppressing the serum PTH level. Another indirect effect of vitamin D on lipid profile is due to vitamin D increasing insulin secretion and sensitivity, thus influencing lipid metabolism. These theories are yet to be proven; however, a few studies have found associations between vitamin D and lipid profiles, especially TG and HDL. Zitterman et al. (2009) reported that low vitamin D was associated with low TG and LDL. This finding has been supported by few other studies (Botella-Carretero et al., 2007; Rejnmark et al., 2010). HDL was found to be inversely associated with vitamin D levels (Botella-Carretero et al., 2007; Fraser et al., 2010; Kevin C. Maki et al., 2009; Oosterwerff et al., 2011). On the other hand, there are some theories that vitamin D supplementation does not affect lipid profiles; instead, statin therapy for hyperlipidaemia might increase the vitamin D level. However, Rejnmark et al. (2010) reported that treatment of simvastatin did not affect vitamin D levels, suggesting instead that vitamin D might influence lipid profile.

2.2.8.4 High blood pressure

Hypertension is a major risk factor for cardiometabolic diseases. Vitamin D appears to have an effect on the suppression of renin-angiotension-aldosterone system activity via its effects on the juxtaglomerular apparatus. This function explains how vitamin D protects against hypertension. In addition, the classical effect of vitamin D on serum PTH suppression has also been found to exert an anti-hypertensive function by suppressing the production of pro-inflammatory cytokines, including tumour necrosis factor (TNF)-alpha. TNF-alpha is known to be implicated in the promotion of arterial stiffness.

Randomized controlled trials have reported that systolic blood pressure (SBP) was significantly reduced in groups receiving vitamin D supplementation compared to

placebo (Pfeifer et al., 2001; Witham, Dove, et al., 2010) . However, no association was found between vitamin D and diastolic blood pressure (DBP). In contrast, Hsia et al. (2007) reported an increase in SBP after two years of intervention, whereas DBP was significantly reduced. A meta-analysis reported no significant reduction in SBP but DBP was reduced significantly (Witham, Nadir, & Struthers, 2009). The same study also reported no significant BP reduction in subjects with baseline BP within the normal range.

2.2.8.5 Metabolic syndrome

By definition, metabolic syndrome is a condition that occurs when three or more metabolic risk factors, such as central obesity, high blood glucose, high cholesterol level, and high blood pressure, exist in one person. As explained earlier, vitamin D deficiency can cause high blood pressure, diabetes mellitus, and hyperlipidaemia. Although vitamin D deficiency does not cause obesity, a few studies have found that vitamin D was inversely associated with obesity. Therefore, vitamin D deficiency may indirectly cause metabolic syndrome. Botella-Carretero et al. (2007) reported a prevalence of metabolic syndrome of almost 70% in morbidly obese vitamin D-deficient participants, with most of the metabolic risk factors determined by low HDL and high TG levels. These results were supported by Oosterwerff et al. (2011), who reported that the association between vitamin D and metabolic syndrome was mainly due to low HDL and high waist circumference.

2.2.9 Vitamin D implications for health-related quality of life (HRQOL)

Quality of life is defined as "the individual's perception of their position in life in the context of the culture and value system in which they live and in relation to their goals, expectation, standards and concerns" (WHO, 1997). HRQOL is based on the individual's perception ofhis well-being, which is of a multi-dimensional order and includes the current health situation, not the disease, and his view of the future. Because

vitamin D deficiency is known to impact individuals' well-being due to musculoskeletal pain and depression, it is thought that vitamin D deficiency may have a negative impact on HRQOL. Many studies have found an association between vitamin D deficiency and HRQOL (Chao, Ekwaru, Ohinmaa, Griener, & Veugelers, 2014; Ecemis & Atmaca, 2013; W. Huang et al., 2013; Motsinger, Lazovich, MacLehose, Torkelson, & Robien, 2012; Witham, Crighton, et al., 2010b).

2.2.9.1 Mental health

WHO defined mental health as a state of well-being in which every individual realizes his or her own potential, can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to her or his community (WHO, 2014). This definition implies that mental health is more than just the absence of mental disorders or disabilities. Social, psychological, and biological factors determine the level of a person's mental health. One of the biological factors that affects mental health is the vitamin D level.

Biologically, calcitriol (1,25(OH)D), which is the active form of vitamin D, is a steroid hormone with potent endocrine, autocrine and paracrine effects induced by binding to the vitamin D receptor (VDR). In general, vitamin D affects mental health through calcitriol, which plays roles inthe synthesis and degradation of several neurotransmitters and in the regulation of several neurotrophic factors.

In terms of neurotransmitters, calcitriol helps increase the availability of dopamine, noradrenaline and adrenaline by activating the gene expression of the enzyme tyrosine hydroxylase (Puchacz, Stumpf, Stachowiak, & Stachowiak, 1996) and increasing the activity of choline acetyltransferase, a key enzyme in acetylcholine synthesis (Sonnenberg, Luine, Krey, & Christakos, 1986). Dopamine, noradrenaline and acetylcholine are the actors in the pathophysiology of mood disorders (Dunlop &

Nemeroff, 2007; M. Humble, 2000), attention deficit or hyperactive disorders, and Alzheimer's disease. Calcitriol is also a potent enhancer of nerve growth factor, which is important for prenatal brain development and is believed to counter act cholinergic system degeneration in Alzheimer's disease (Cattaneo, Capsoni, & Paoletti, 2008). Vitamin D deficiency may result in changes in the endocrine system, which may potentially interfere with diverse brain functions related to mental health (Eyles et al., 2009). Therefore, based on this evidence, it can be concluded that vitamin D plays an important role in mental health.

Nevertheless, few studies have examined the association between vitamin D and overall mental health. Only a few studies have found that vitamin D deficiency was associated with mental health (Anand et al., 2011; Ecemis & Atmaca, 2013; W. Huang et al., 2013; Motsinger et al., 2012; Ohta, Uemura, et al., 2014; Sakalli et al., 2012).

However, there have been many studies on vitamin D and depression (Jorde, Sneve, Figenschau, Svartberg, & Waterloo, 2008; Jozefowicz, Rabe-Jablonska, Wozniacka, & Strzelecki, 2014; Kjaergaard et al., 2012; Kwasky & Groh, 2014; May et al., 2010; Miyake, Tanaka, Okubo, Sasaki, & Arakawa, 2015; Moran, Teede, & Vincent, 2014; Penckofer, Kouba, Byrn, & Estwing Ferrans, 2010; Polak, Houghton, Reeder, Harper, & Conner, 2014). In a recent review of data from both cross-sectional and cohort studies, serum 25(OH)D levels were found to be inversely associated with the risk of depression (Ju, Lee, & Jeong, 2013). However, a meta-analysis by Shaffer et al. (2014) found that vitamin D supplementation had no significant effect on depressive symptoms for subjects without clinically significant depression. The same study, however, showed that vitamin D supplementation led to a moderate significant improvement in participants with clinical depressive disorder. Nevertheless, many observational studies have found an association between vitamin D and depression in the general population (Chao et al., 2014; Jorde et al., 2008; Kjaergaard et al., 2012) as well as biological

plausibility of a role for vitamin D in depression. Therefore, vitamin D supplementation for the prevention of depressive symptoms in individuals without depression is not justified.

2.2.9.2 Physical health

Proximal muscle weakness is a prominent feature of the clinical symptoms of vitamin D deficiency. One possible underlying mechanism is a decrease in vitamin D levels, which may lead to decreased VDR expression, thus causing down regulation of VDR function. Over time, this down regulation would progressively reduce the type II fibres in muscles due to impaired muscle cell protein synthesis (Boland, 1986), thus causing sarcopenia. Sarcopenia is associated with greater risk of disability and falls, especially among the elderly. Ageing is a risk factor for vitamin D deficiency; therefore, it is possible that falls in the elderly could be due to sarcopenia resulting from vitamin D deficiency. In addition to vitamin D deficiency, VDR expression in human muscle tissue was found to decrease with age (H. A. Bischoff-Ferrari et al., 2004). A review by Girgis, Clifton-Bligh, Turner, Lau, and Gunton (2014) found that although observational studies have found associations between vitamin D deficiency and falls, muscle weakness and sarcopenia in the elderly, clinical trials have revealed inconsistent results. However, the review supported the benefits of vitamin D supplementation in populations at risk of vitamin D deficiency, such as the elderly, to reduce falls. Falls and fractures may be prevented by a vitamin D intake of at least 700 to 800 IU per day and a target serum 25(OH)D level of 75 nmol/l (30 ng/ml) for optimal lower extremity strength and bone health (Heike A. Bischoff-Ferrari, 2007).

In addition to proximal muscle weakness, several cross-sectional studies have reported that vitamin D deficiency was associated with non-specific musculoskeletal pain (Heidari, Shirvani, Firouzjahi, Heidari, & Hajian-Tilaki, 2010; Hicks et al., 2008; Knutsen, Brekke, Gjelstad, & Lagerlov, 2010; Matossian-Motley, Drake, Samimi,

Camargo, & Quraishi, 2014; McBeth et al., 2010). The mechanism underlying the association between musculoskeletal pain and vitamin D status is likely osteomalacia. It is known that vitamin D deficiency causes a reduction in calcium absorption in the intestines, which results in both increased PTH levels and increased in osteoclast activity. Osteomalacia is the end results of this PTH-mediated process as are osteopenia and osteoporosis (Holick, 2003). In addition, increased PTH due to prolonged vitamin D deficiency may also induce phosphaturia, which leads to a reduction in calcium-phosphate interaction and consequently bone mineralization. The resultant matrix hydrates and expands, causing the subperiosteal space to become oedematous and thus producing unrelenting aching sensation in the bones (Holick, 2003). Many studies have found benefits of vitamin D supplementation for musculoskeletal pain (Abbasi, Hashemipour, Hajmanuchehri, & Kazemifar, 2013; W. Huang et al., 2013; Le Goaziou et al., 2013; Le Goaziou et al., 2014; Malabanan et al., 1998; Schreuder et al., 2012). Other studies, however have found vitamin D did not relieve bone pain or musculoskeletal pain (Knutsen et al., 2014; Warner & Arnspiger, 2008).

2.2.10 Prevention of vitamin D deficiency

2.2.10.1 Sunlight exposure

UVB from sunlight is the primary source of vitamin D and the most straight forward approach to vitamin D deficiency prevention. Sensible exposure to sunlight between 10.00 am and 3.00 pm for not more than five to ten minutes per day (Holick, 2011) results in sufficient vitamin D synthesis by the epidermis. However, this recommendation depends on the season of the year, latitude, age of the individual, clothing style and degree of skin pigmentation, which may influence the amount of UVB light that reaches the skin for vitamin D synthesis. An adult exposing more than 90% of their skin to UVB light is equivalent to taking a single-dose vitamin D supplement of approximately 15,000 to 20,000 IU (Holick, 2011). However, individuals

with Fitzpatrick skin type 4 may need a five- to ten-fold longer exposure compared to those with skin type 1. Similarly, elderly people aged more than 70 years produce four-fold less vitamin D from photosynthesis compared to younger individuals, which means the elderly need longer exposure to sunlight. Nevertheless, excessive sunlight exposure does not cause vitamin D intoxication because all of the excess vitamin D and pre-vitamin D3 from vitamin D photosynthesis is photolysed to biologically inactive photo products (Holick, 2006).

Concerns about health detriment due to excessive sunlight exposure make it difficult to recommend sunlight exposure as the primary means of preventing vitamin D deficiency. There is no question that excessive sunlight exposure increases the risk of skin cancer, particularly melanoma. The risk of skin cancer is increased in individuals with Fitzpatrick skin types 1 and 2 (Fitzpatrick, 1988). However, Lee H.Y. et al. (2012) reported that skin cancer is a rarer disease in Asians compared to Caucasians, even in Asians with Fitzpatrick skin type 2. Nurbazlin et al. (2013) reported that the UV index was the main factor influencing vitamin D status in Malaysian women. Therefore, health education on sunlight exposure for the prevention of vitamin D deficiency is important because the risk of skin cancer due to excessive sunlight exposure is rare in Malaysians. A study by Istiany et al. (2012) indicated that nutrition education and sunlight exposure, as well as vitamin D supplementation, were needed to increase the serum 25(OH)D concentration in Malaysian women.

In addition to sunlight, sunbeds represent a commercially available source of UVB light. A study by Porojnicu et al. (2008) on the effects of commercial sunbeds found that sunbed use twice per week significantly increased serum 25(OH)D by 40%. However, this method of prevention is not cost effective and is impractical for primary prevention at the population level.

2.2.10.2 Dietary intake

a) Natural sources

Very few foods contain vitamin D naturally. These include cod fish, salmon, egg yolk, milk, mushrooms, and fish liver oil (Table 1.1). However, these foods are not commonly consumed in Malaysian diets. Obtaining a sufficient amount of vitamin D from a regular diet is often problematic for individuals whose diet does not include foods that are naturally rich in vitamin D. Therefore, it is difficult to improve vitamin D status via natural sources alone.

b) Food fortification

Food fortification is one of the best choices to improve the population's vitamin D status through dietary intake. Food fortification is a food-based approach to optimize nutrition where there is a dependence on staples and a narrow range of foods. This approach has the dual advantages of being able to deliver nutrients to larger segment of the population and not requiring radical changes infood consumption patterns. However, the food vehicles used for nutrient fortification need to be well selected so that they can reach the target population. Dairy products, such as milk and margarine, are usually the food of choice for vitamin D fortification. In some countries, such as Canada, Malaysia, Indonesia and the Philippines, vitamin D fortification of margarine is mandated by law.

Vitamin D fortification tends to be beneficial for improving serum 25(OH)D concentrations (O'Donnell et al., 2008). Many studies have shown positive effects of vitamin D fortification of milk, margarine and orange juice on populations' vitamin D status (Khadgawat et al., 2013; Laaksi et al., 2006; Piirainen, Laitinen, & Isolauri, 2007; Tangpricha et al., 2003). However, dairy products and orange juice are not commonly consumed by Malaysians.

Currently in Malaysia, vitamin D fortification of margarine products is mandated by law,yet the prevalence of vitamin D deficiency is still high. Yang et al. (2013) reported that vitamin D-fortified vegetable oil may potentially contribute to a significant daily intake of vitamin D by the whole population. The same study estimated that for Malaysia, where the average oil consumption is 16.1 g/day, vitamin D-fortified vegetable oil at a level of 7.5 to 10 μg/100 g could provide 9.7% to 12.9% of the Institute of Medicine's Estimated Average Requirement of vitamin D. According to a study by Heaney et al. (2003), 1 μg of ingested vitamin D can increase serum 25(OH)D by 1 to 1.2 nmol/l. Therefore, if 10 μg/100 g of vitamin D were used to fortify vegetable oil, we could expect an increase of 10 to 12 nmol/l in the serum 25(OH)D concentration ofthe population. Vegetable oil may be the most suitable vehicle for vitamin D fortification compared to the current practice of mandatory fortification of margarine in Malaysia.

Nevertheless, a study by Vatanparast et al. (2010) reported that despite mandatory food fortification with vitamin D in Canada, vitamin D intake levels are still inadequate in children. Similar findings were reported in Finland where vitamin D fortification of milk did not improve the vitamin D status in young Finnish men (Valimaki, Loyttyniemi, & Valimaki, 2007). This findings could be due to the presence of only 100 IU of vitamin D per 8 oz. of fortified milk, which means that an individual needs to drink at least six glasses of milk per day, which is not realistic. Therefore, consideration should be given to strategies to improve vitamin D intake by increasing both the amount of vitamin D added to foods and the range of foods eligible for fortification.

2.2.10.3 Vitamin D supplementation

It is difficult to achieve the RNI of 600 IU to 800 IU per day for adults aged 18 to 50 years, as suggested by the IOM (Ross et al., 2011), using natural food or fortified food alone. In addition, this recommendation is only for skeletal health benefits. It is

unknown whether this dosage is sufficient to provide all of the potential non-skeletal health benefits associated with vitamin D, such as those associated with diabetes or CVD risk factors.

The Clinical Practice Guidelines of the Endocrine Society suggest that adults should take a vitamin D supplement of at least 1500 to 2000 IU per day to maintain a serum 25(OH)D level greater than 75 nmol/l (Holick et al., 2011). This recommendation is for the maintenance of vitamin D levels but not for the treatment of vitamin D deficiency. The Endocrine Society recommended 50,000 IU of vitamin D once per week for eight weeks, which is equivalent to 7143 IU per day, for the treatment of vitamin D deficiency (Holick et al., 2011). This regimen is intended to increase the serum 25(OH)D concentration from a deficient level to above 50 nmol/l which is followed by maintenance therapy of 1500 to 2000 IU per day to prevent the recurrence of vitamin D deficiency. According to Holick et al. (2011), at least 1500 to 2000 IU/day of vitamin D supplementation is required to consistently maintain the serum 25(OH)D level above 75 nmol/l. The consumption of 50,000 IU of vitamin D is considered a safe dose as the tolerable upper intake level of vitamin D is 10,000 IU per day (Ross et al., 2011). However, overweight and obese adults or patients with malabsorption syndromes may need at least two- or three-fold greater doses of vitamin D (6000 to 10,000 IU per day) for the treatment and prevention of vitamin D deficiency.

Nevertheless, many RCTs provide less than 1000 IU per day of vitamin D supplements as the intervention in studies that aim to evaluate the non-skeletal effects of vitamin D (Hsia et al., 2007; Major et al., 2007; Patel, Poretsky, & Liao, 2010; Pfeifer et al., 2001; Pittas, Harris, et al., 2007). Wood et al. (2012) found that participants provided 400 IU of vitamin D3 supplements daily had a mean serum level of 25(OH)D lower than 75 nmol/l, compared to participants provided 1000 IU of

vitamin D supplements daily, who had mean serum 25(OH)D greater than 75 nmol/l after 12 months of treatment (Pfeifer et al., 2001).

Vitamin D comes in two forms, vitamin D2 and vitamin D3, as mentioned in section 2.2.1. Both vitamin D2 and vitamin D3 are commonly used for food fortification and in vitamin D supplements. However, there is concern that vitamin D2 may not be bio-equivalent to vitamin D3 and may be less effective than vitamin D3. Nevertheless, Holick et al. (2008) found that a daily dose of 1000 IU of vitamin D2 was as effective as 1000 IU vitamin D3 in maintaining serum 25(OH)D levels. Therefore, both forms of vitamin D can be used in supplements and food fortification.

2.3 Systematic review on the effect of vitamin D supplementation on cardiometabolic risk factors

Bibliographic databases including EMBASE, PubMed and the Cochrane Library (Cochrane Central Register of Controlled Trials) were used in the search strategy, which was limited to English-language articles. The keywords used were based on the research question, which is "What is the effect of vitamin D supplements on cardiometabolic risk factors in vitamin D-deficient premenopausal women?". Although the research question in this study were confined to premenopausal women, the terms used in the search included all age ranges of the adult population and both males and females due to limited studies on vitamin D in premenopausal women, especially studies on cardiometabolic risk. Cardiometabolic risk referred to the presence of insulin resistance, high blood pressure, high blood glucose, overweight or obesity or the presence of low HDL cholesterol, high LDL cholesterol or high TG levels, as defined by Brunzell et al. (2008). Regarding the definition of vitamin D deficiency, because there is no consensus on this definition, all studies that measured vitamin D status were included in this review.

The search terms used for this review were as follows: (adults OR (age 35 and above) OR middle aged OR elderly) AND (vitamin D OR (serum 25(OH)D)) AND ((cardiometabolic) OR (cardiovascular) OR (metabolic syndrome) OR (diabetes mellitus OR high blood glucose) OR (hypertension OR high blood pressure) OR ((hyperlipidaemia) OR (high lipid profile) OR (high triglycerides) OR (high LDL) OR (low HDL)). The titles and abstracts of studies conducted before the year 2000 were scanned to identify potential articles for review. Potentially eligible papers were retrieved in hard copy for a more detailed review.

Studies of vitamin D effects on non-musculoskeletal health, such as cardiovascular disease, hyperlipidaemia, and diabetes mellitus, are considered rather new and novel. Most studies were conducted in Western countries, especially those at northern latitudes. Very few studies were conducted in Asian countries because it was once thought that only people living at northern latitudes had vitamin D deficiency. Therefore, this systematic review is intended to achieve a better understanding of the effects of vitamin D on cardiometabolic risk factors. A flow chart of the search strategy is presented in Figure 2.3. A total of 30 articles were included in this review.

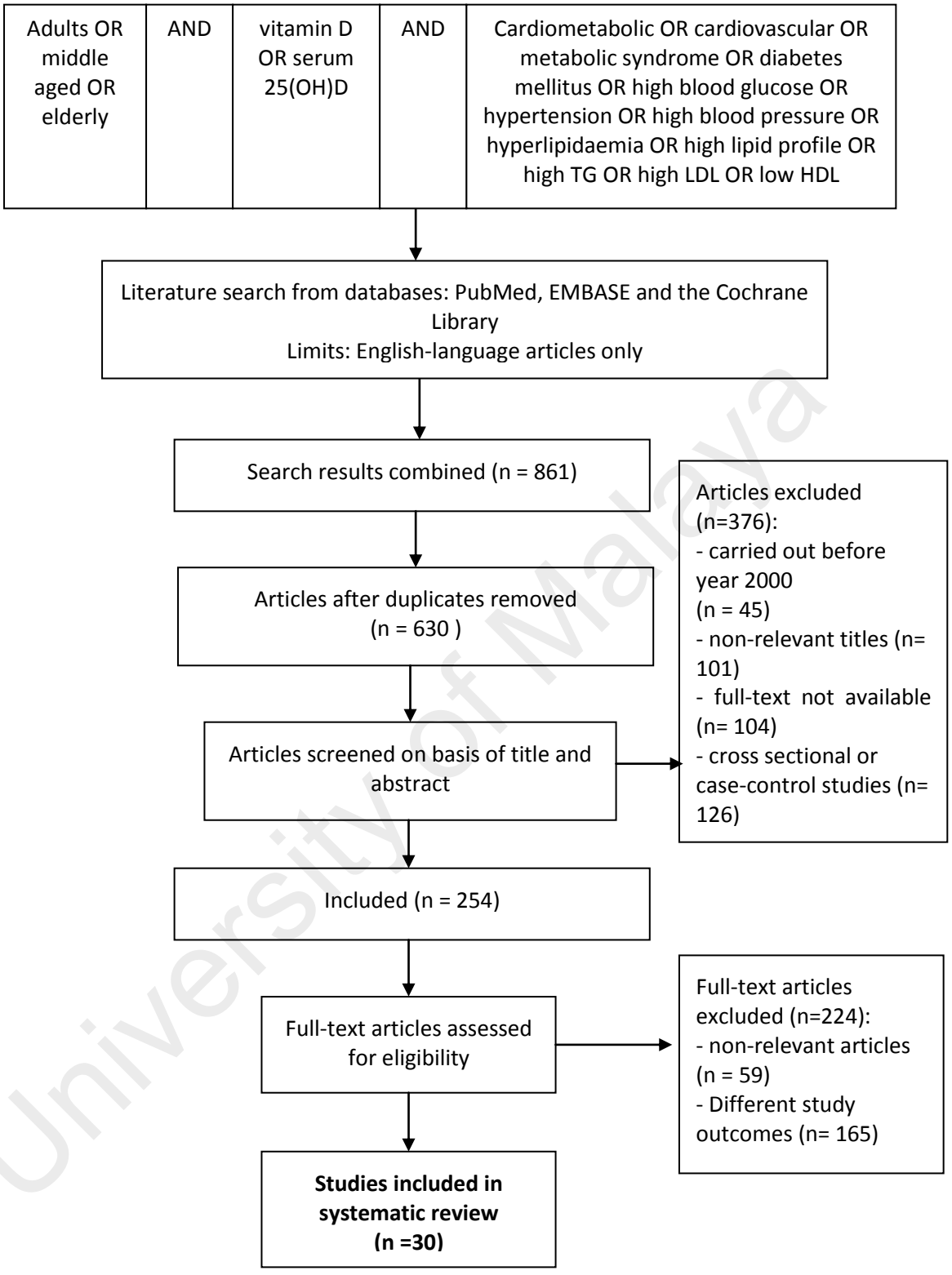

Figure 2.3: Flow chart of search strategy for studies on vitamin D impact on cardiometabolic risk factors

The risk of bias of RCTs was assessed using a modified version of the Cochrane Collaboration's tool (J.P.T. & S., 2009). The items included random sequence generation, allocation concealment, blinding of participants, blinding of self-reported outcome assessment, blinding of objective outcome assessment, completeness of outcome data, and selective reporting. For observational studies, the quality of reporting was assessed using the STROBE checklist (von Elm et al., 2008).

The studies with reporting quality scores under 13 or with insufficient temporal information between exposure and outcome were considered to be of low quality. The remaining studies were arbitarily categorized into high grade quality scores.

2.3.1 Demographics characteristics

Table 2.5 summarizes the studies selected for this systematic review. Thirty articles with six cohort studies (Al-Daghri, Alkharfy, Al-Saleh, et al., 2012; Aljabri et al., 2010; Gagnon et al., 2012; Tai et al., 2008; Talaei et al., 2013; T. J. Wang et al., 2008) and 24 RCTs (Al-Daghri et al., 2014; Asemi et al., 2014; Breslavsky et al., 2013; Gepner et al., 2012; Harris, Pittas, & Palermo, 2012; Hsia et al., 2007; Jorde et al., 2008; Major et al., 2007; K. C. Maki et al., 2011; Mitri et al., 2011; Nagpal et al., 2009; Patel et al., 2010; Pfeifer et al., 2001; Pittas, Harris, et al., 2007; Salehpour et al., 2013; Sollid et al., 2014; Strobel et al., 2014; Tzotzas et al., 2010; von Hurst et al., 2010; Witham, Crighton, et al., 2010a; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009) were identified. The studies were conducted in adult populations aged more than 18 years, type 2 diabetic patients, overweight and obese individuals, postmenopausal and premenopausal women and osteoporotic patients.

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Salehpour et al. (2012)	Double-blind, RCT n = 85 Healthy, overweight and obese Aged 18 – 50 years	ELISA	BP Lipid profile ApoA-I	Daily 25 µg Vitamin D3 (1000 IU) or placebo	Tehran, Iran November 12 weeks	Improved HDL, ApoA-I and LDL:ApoB-100 ratio Increases LDL and TC	Sunlight exposure, diet, physical activity and seasonal history not assessed
Strobel et al. (2014)	Double-blind RCT n = 86 Aged 18 to 80 years Type 2 DM women	RIA	Blood glucose Blood insulin HOMA-IR BP	Weekly 20 drops Vigantol oil (1904 IU) or placebo oil for 6 months	Frankfurt, Germany 12 months	HbA1c significantly improved	Sunlight, physical activity, diet and seasonal history not assessed
K. C. Maki et al. (2011)	Double-blind RCT n = 60 Age 18 to 79 years Centrally obese man and women	ECLIA	Lipid profiles BP CRP	Daily MVM + 1200 IU vitamin D or MVM for 8 weeks	Illinois, US 8 weeks	Dosage of vitamin D was inadequate to increase level to > 75 nmol/l	All potential confounders were assessed. Using MVM
Wood et al. (2012)	Double-blind RCT n = 305 Healthy Caucasian post-menopausal women	Not mentioned	Lipid profiles ApoA-1 HOMA h-CRP BP	Daily 400 IU or 1000 IU or placebo	Aberdeen, UK 12 months	Found significant seasonal effects on BP	Using UVB light-sensitive badges to measure sunlight exposure Potential confounders assessed
Breslavsky et al. (2013)	Double-blind RCT n = 47 type 2 DM	CPBA	Lipid profiles HOMA-IR h-CRP BP	Daily 1000 IU vitamin D3 or placebo	Israel 12 months	Significant improvement in arterial stiffness in diabetic patient was found	Large number of dropped-out Potential confounders not assessed

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors (continued)

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Nagpal et al. (2009)	Double-blind RCT n = 100 Healthy, centrally obese, male Age ≥ 35 years	RIA	OGIS index Insulin secretion Lipid profiles BP	3 doses of 120,000 IU vitamin D fortnightly or placebo	New Delhi, India 6 weeks	Improved 3h-OGIS	26% dropped-out Male only Vitamin D deficient at baseline
Witham, Crighton, et al. (2010b)	Double-blind RCT n = 61 Type 2 DM Aged ≥ 18 years	RIA	Endothelial function IR BP	Single dose of 100,000 IU vitamin D3 or 200,000 Vitamin D3 or placebo	Dundee, UK 16 weeks	SBP fall significantly No changes in renin or aldosterone levels was found	On ACE and statin Abnormal baseline SBP
Hsia et al. (2007)	Double-blind RCT n = 36,282 Postmenopausal women from the Women's Health Initiative trials Aged 50 to 79 years	Not mentioned	CVD events Intermediate outcomes: BP, Lipid profiles	Twice daily of 200 IU vitamin D with 500 mg calcium or placebo	US 2 years for intermediate outcomes 7 years for CVD event outcomes	No changes in CVD events SBP increased DBP reduced LDL increased	Bioassays performed to only 6% subjects at 2 years Allowed to take own calcium supplements Design to evaluate fracture
Zitterman et al (2009)	Double-blind RCT n = 200 Healthy overweight Aged 18 to 70 years	RIA	Weight loss BP Lipid profiles Glucose h-CRP	Daily 1000 IU vitamin D or placebo	Germany 12 months	Increased LDL Decreased 13.5% TG	Weight-loss program

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors (Continued)

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Harris et al. (2012)	RCT n = 89 Overweight and obese, pre-diabetes (FBS ≥5.5 mmol/l or HbA1c ≥ 5.8% and < 7%)	RIA	Insulin secretion Insulin sensitivity glycaemia	Daily 4000 IU vitamin D or placebo (asked to take with daily 600 mg Ca)	Boston, US 12 weeks	Insulin sensitivity decreased by 4% Insulin secretion increased 12 %	Insulin sensitivity in placebo increase by 12% - caused the significant difference
Pfeifer et al. (2001)	Double-blind RCT n = 148 women age ≥ 70 years	RIA	BP PTH	Twice a day of 600 mg Ca or 600 mg Ca with 400 IU Vitamin D	Germany 80weeks	Decreased PTH Decreased SBP	Vitamin D deficient and SBP abnormal at baseline
Gepner et al. (2012)	Double-blind RCT n = 114 postmenopausal women with se 25(OH)D between 20 to 150 nmol/l	HPLC	Brachial artery FMD BP Glucose Lipid profiles CRP	Daily 2500 IU vitamin D cookie or placebo cookie	Wisconsin, US 4 months	All not significant	Generally healthy participants with normal baseline
Asemi et al. (2014)	Double-blind RCT n = 104 aged 18 to 40 years Healthy overweight and obese vitamin D deficient (< 50 nmol/l) with PCOS	ELISA	HOMA-IR Glucose Lipid profiles	- 1000 mg/d Ca + vitamin D placebo - 50,000 IU/wk Vitamin D + Ca placebo - 1000 mg/d Ca + 50,000/wk vitamin D - placebo	Kashan, Iran 8 weeks	Decreased in HOMA-IR, insulin levels, TG and VLDL	Premenopausal women Abnormal HOMA-IR at baseline

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors (Continued)

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Major et al. (2007)	Double-blind RCT n = 63 Overweight or obese premenopausal women	Not mentioned	Weight loss Glucose BP	Daily 600 mg Ca + 200 IU vitamin D or placebo	Quebec City, Canada 15 weeks	Decreased Total:HDL, LDL, LDL:HDL	Subjects on weight-loss program
Mitri et al. (2011)	2-by-2 factorial design, double-blind RCT n = 92 Aged ≥ 40 years, overweight or obese with glucose tolerance or early diabetes	LC-MS/MS	Disposition index Insulin sensitivity glucose	vitamin D 2000 IU/d or placebo or Ca 400 mg/twice daily	Boston, US 16 weeks	Vitamin D improved disposition index and insulin secretion by 26%	High retention rate Used disposition index (sensitive to measure β-cells function)
Ryu et al. (2013)	Double-blind RCT n = 158 type 2 DM with vitamin D deficient (< 50 nmol/l)	ECLIA	Glucose HOMA-IR HbA1c	1000 IU vitamin D + 100 mg Ca twice daily or 100 mg Ca only	Chuncheon and Seoul, Korea 24 weeks	All changes were not significant	Only 43% in intervention group reach serum 25(OH)D > 80 nmol/l
Pittas, Harris, et al. (2007)	Double-blind RCT n = 314 Healthy osteoporotic aged ≥ 65 years	CPBA	Blood glucose HOMA-IR	Daily 700 IU vitamin D and 500 mg Ca or placebo	Boston, US 3 years	Intervention prevented the HOMA-IR and blood glucose increment in IFG subjects compared to placebo	No changes in NFG subjects

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors (Continued)

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Jorde et al. (2008)	Double-blind RCT n = 438 aged 21 to 70 years, healthy, overweight and obese	RIA	Lipid profiles BP OGTT HOMA	40,000 IU vitamin D or 20,000 IU vitamin D or placebo plus 500 mg Ca for both group	Norway 1 year	All changes were not significant	Serum 25(OH)D at baseline > 50 nmol/l Main objective were weight loss
Yiu et al. (2013)	Double-blind RCT n = 100 Type 2 DM with se 25(OH)D < 75 nmol/l	ELISA	FMD BP Lipid profile h-CRP Blood glucose	5000 IU/d vitamin D or placebo	Hong Kong 12 weeks	All changes were not significant	Mean baseline se 25(OH)D > 50 nmol/l
von Hurst et al. (2010)	Double-blind RCT n = 235 Women of South Asian origin with vit D deficient (< 50 nmol/l) and insulin resistant	RIA	Insulin sensitivity	4000 IU/d vitamin D or placebo	New Zealand 6 months	Insulin sensitivity improved only when se 25(OH)D > 80 nmol/l	Vitamin D deficient with insulin resistant at baseline but non-diabetic Problem with compliance to treatment
Sollid et al. (2014)	RCT n = 556 aged 21 to 80 years pre-diabetic	LC-MS/MS	Blood glucose HOMA-IR Lipid profiles h-CRP BP	20,000 IU vitamin D3 or placebo per week	Norway 1 year	All changes were not significant	Part of on-going 5 year RCT Low no of subjects with vitamin D deficiency at baseline

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors (Continued)

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Al-Daghri et al. (2014)	RCT n = 120 aged 30 to 70 years with known type 2 DM	ELISA	HOMA-IR Blood glucose BP Lipid profiles	Daily 2000 IU vitamin D3 or placebo	Saudi Arabia 18 months	Improvement in LDL, HOMA-IR In females: improved in LDL, HOMA-IR, HOMA β%	23.3% attrition rate
Patel et al. (2010)	RCT n = 24 vitamin D deficient (< 62.5 nmol/l) and on stable diabetes treatment	Not mentioned	Insulin sensitivity Lipid profiles	Daily 400 IU or 1200 IU of vitamin D	New York, US 4 months	No improvement in all study outcomes	Pilot study Normal lipid profiles and blood glucose at baseline
Tzotzas et al. (2010)	Pilot RCT n = 44 Obese women	ECLIA	HOMA index Lipid profiles ApoA1 ApoB	Dietary intervention	Greece 20 week	10% weight loss was associated with 34% increased serum 25(OH)D; Serum 25(OH)D inversely associated with IR index	Weight-loss intervention program
Aljabri et al. (2010)	Cohort study n = 80 aged 12 years with type 1 DM, vitamin D deficient	CBPA	Glycosylated haemoglobin	4000 IU/d vitamin D3 with 1200 mg/d Ca	Saudi Arabia 12 weeks	No improvement in all study outcomes	Study design non-RCT Adolescent Subjects are more likely to achieved lower glycosylated haemoglobin if had higher vitamin D status

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors (Continued)

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Gagnon et al. (2012)	Population based cohort study n = 4164 Aged > 25 years without MetS at baseline	RIA	MetS and its components	None	Australia 5 years	For each 25 nmol/l decreased in 25(OH)D, the risk of developing MetS at five year increased by 23% Serum 25(OH)D < 57.5 nmol/l associated with 74% increased risk of developing MetS in five year Low serum 25(OH)D associated with higher WC, blood glucose, TG and HOMA-IR	No comparison group No vitamin D intervention No data on PTH Participants had lower MetS risk profile with higher se 25(OH)D at baseline
T. J. Wang et al. (2008)	Cohort study n = 1739 Framingham Offspring cohort	RIA	BP DM	None	Boston, US 7.6 years	Prevalence < 37.5 nmol/l = 28%; < 25 nmol/l = 9%; Risk of developing CV is increased to 2-fold in subjects with vitamin D < 37.5 nmol/	Part of Framingham Offspring cohort study Diet and physical activity history taken PTH not measured

Table 2.5: Summary of the studies selected for systematic review on the effect of vitamin D on cardiometabolic risk factors (Continued)

Author	Study design, study population	Serum 25 (OH)D assay	Study outcomes	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Talaei et al. (2013)	Cohort study, Single-blind n = 100 30 to 70 years with type 2 DM	RIA	HOMA-IR Blood glucose	50,000 IU vitamin D weekly	Iran 8 weeks	Improvement in blood glucose, insulin and HOMA-IR	No comparison group On diabetic medication
Tai et al. (2008)	Cohort study n = 37 Aged 19 to 75 years with vitamin D deficiency (< 50 nmol/l), no DM	RIA	Glucose Insulin sensitivity	2 oral doses of 100,000 IU vitamin D 2 week apart	Adelaide, Australia 4 weeks	All changes were not significant	Non-diabetic at baseline
Al-Daghri et al. (2012)	Cohort study n = 59 Aged 18 to 65 years, non-diabetic	ELISA	MetS and its components	Sunlight exposure advice 5 to 30 min twice a week and vitamin D-rich diet advice	Saudi Arabia 12 months	Decreased prevalence of MetS, low HDL and High TG	Health education program intervention. No comparison group

2.3.2 Types of intervention

Nineteen RCTs were conducted to observe the effect of vitamin D on cardiometabolic risk factors compared with placebo (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Asemi et al., 2014; Breslavsky et al., 2013; Gepner et al., 2012; Harris et al., 2012; Hsia et al., 2007; Jorde et al., 2008; Major et al., 2007; Mitri et al., 2011; Nagpal et al., 2009; Pittas, Harris, et al., 2007; Salehpour et al., 2012; Sollid et al., 2014; Strobel et al., 2014; von Hurst et al., 2010; Witham, Dove, et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009), two RCTs compared vitamin D with calcium supplementation (Pfeifer et al., 2001; Ryu et al., 2013), and one study compared vitamin D with multivitamin (K. C. Maki et al., 2011). Eight RCTs used a combination of vitamin D supplements with calcium as active supplements (Aljabri et al., 2010; Harris et al., 2012; Hsia et al., 2007; Jorde et al., 2008; Major et al., 2007; Pfeifer et al., 2001; Pittas, Harris, et al., 2007; Ryu et al., 2013), whereas 14 studies used only vitamin D supplements as active supplements (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Breslavsky et al., 2013; Gepner et al., 2012; Mitri et al., 2011; Nagpal et al., 2009; Patel et al., 2010; Sollid et al., 2014; Tai et al., 2008; Talaei et al., 2013; von Hurst et al., 2010; Witham, Dove, et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009). One study was conducted using a combination of either calcium and vitamin supplements, vitamin D supplements only, calcium supplements only or placebo (Asemi et al., 2014). Among the cohort studies, two used vitamin D supplements only as the intervention (Tai et al., 2008; Talaei et al., 2013). Two studies provided sunlight exposure or dietary advised as the intervention (Al-Daghri, Alkharfy, Al-Saleh, et al., 2012; Tzotzas et al., 2010).

2.3.3 Study design

In this systematic review, 24 (80%) of the studies selected used a randomized controlled trial design (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Asemi et al.,

2014; Breslavsky et al., 2013; Gepner et al., 2012; Harris et al., 2012; Hsia et al., 2007; Jorde et al., 2008; Major et al., 2007; K. C. Maki et al., 2011; Mitri et al., 2011; Nagpal et al., 2009; Patel et al., 2010; Pfeifer et al., 2001; Pittas, Harris, et al., 2007; Ryu et al., 2013; Salehpour et al., 2012; Sollid et al., 2014; Strobel et al., 2014; Tzotzas et al., 2010; von Hurst et al., 2010; Witham, Dove, et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009), whereas six (20%) used a cohort study design (Al-Daghri, Alkharfy, Al-Saleh, et al., 2012; Aljabri et al., 2010; Gagnon et al., 2012; Tai et al., 2008; Talaei et al., 2013; T. J. Wang et al., 2008) to assess the effects of vitamin D on cardiometabolic risk factors. Out of 24 RCTs, one study was conducted with a 2-by-2 factorial study design (Mitri et al., 2011) and one was a pilot study (Tzotzas et al., 2010).

2.3.4 Quality assessment

Judgements about each risk of bias item assessed using the Cochrane risk of bias tools for 24 RCT studies are presented in Figures 2.4 and 2.5. The study quality ranged from fair to good. Only a few items were judged to have a high risk of bias. Most items were judged to have a low risk of bias, but some items were judged to have an unclear risk of bias, which was mainly the result of insufficient reporting.

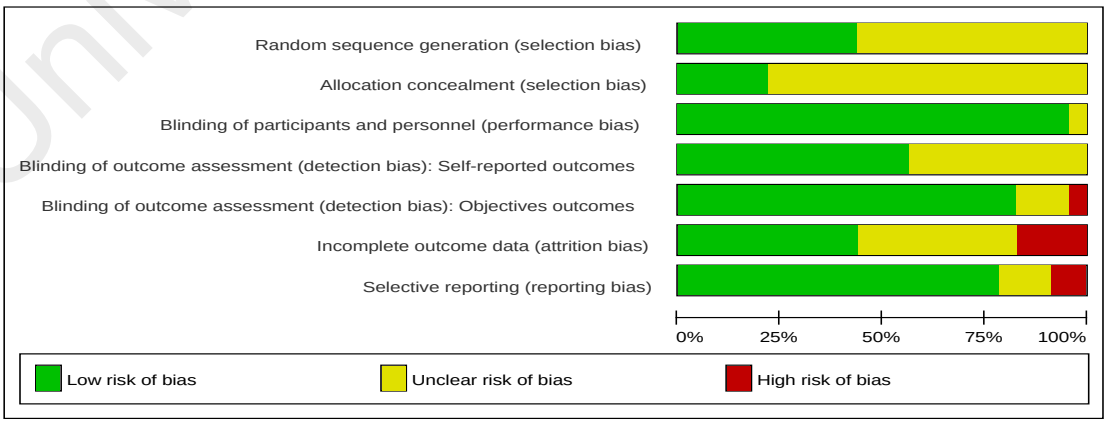

Figure 2.4: Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

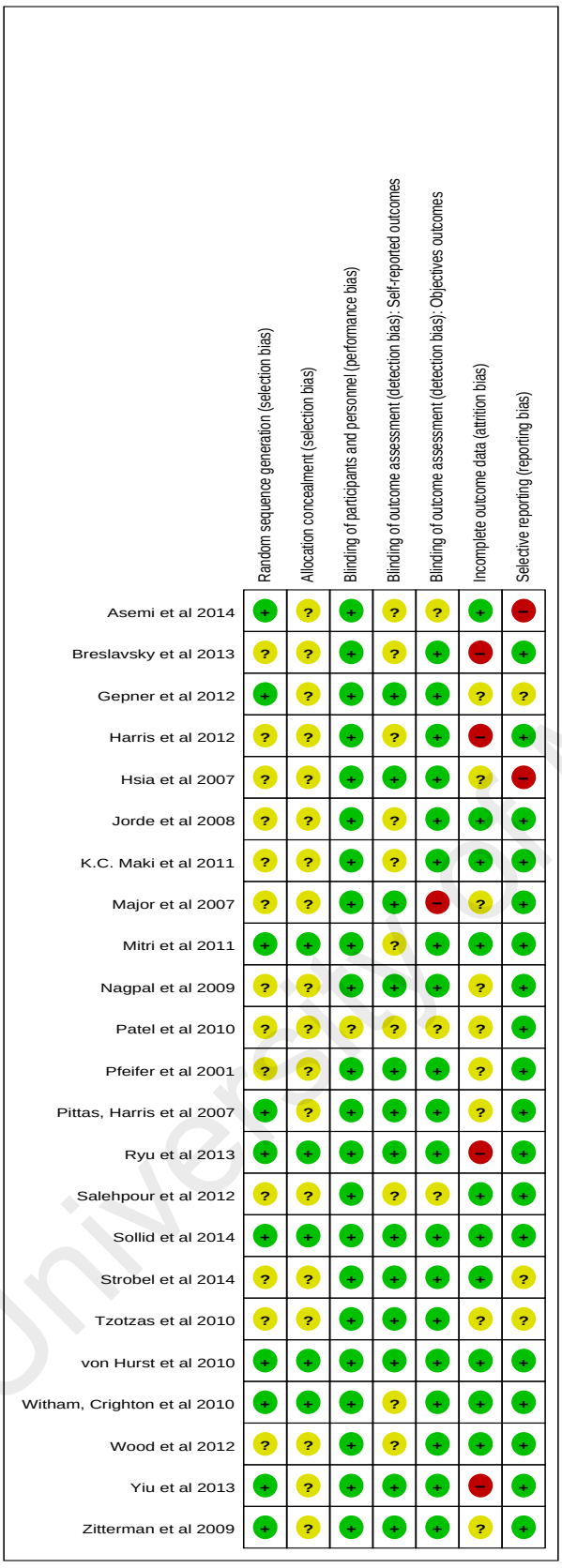

Figure 2.5: Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

Randomization was performed in all of the included studies; however, only 10 studies appropriately described the sequence of generation (Asemi et al., 2014; Gepner et al., 2012; Mitri et al., 2011; Pittas, Harris, et al., 2007; Ryu et al., 2013; Sollid et al., 2014; von Hurst et al., 2010; Witham, Dove, et al., 2010; Yiu et al., 2013; A. Zittermann et al., 2009). Most of the studies did not mention allocation concealment, except for five trials (Mitri et al., 2011; Ryu et al., 2013; Sollid et al., 2014; von Hurst et al., 2010; Witham, Dove, et al., 2010). In most of the included RCTs, the participants and personnel were blinded, corresponding to a low risk of bias. Two studies reported a loss to follow-up of more than 20% of the baseline number of participants (Jorde et al., 2008; Nagpal et al., 2009) and the remaining 25 studies reported losses to follow-up of less than 20%. Financially, study protocols were supported partly (Nagpal et al., 2009; Ryu et al., 2013; Sollid et al., 2014; Strobel et al., 2014; von Hurst et al., 2010; Yiu et al., 2013) or solely (K. C. Maki et al., 2011; Pfeifer et al., 2001) by pharmaceutical companies that produce and market the vitamin D under consideration in each study. Funding sources were not reported in the disclosures of four study protocols (Breslavsky et al., 2013; Mitri et al., 2011; Tzotzas et al., 2010; A. Zittermann et al., 2009), thus providing insufficient information to permit judgement, whereas other studies was funded by university or government grants (Harris et al., 2012; Hsia et al., 2007; Jorde et al., 2008; Major et al., 2007; Patel et al., 2010; Pittas, Harris, et al., 2007; Witham, Dove, et al., 2010; Wood et al., 2012).

Seven of the observational studies included in this systematic review were assessed for quality of reporting using the STROBE checklist (Table 2.6). Only four of the seven justified their sample size by describing sample size calculations (Al-Daghri, Alkharfy, Al-Saleh, et al., 2012; Gagnon et al., 2012; Tai et al., 2008; Talaei et al., 2013). Only one study (Gagnon et al., 2012) adequately reported non-participation at each stage and used a flow diagram to represent the numbers of participants. The same study also

described how missing data were addressed statistically, whereas the others did not mention how they handled missing data.

One out of six of the selected papers did not provide a clear account of the outcomes, exposures, effect modifiers and confounders under assessment (Aljabri et al., 2010). Low reporting of the statistical methods used to address confounding was observed in three studies (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Al-Daghri, Alkharfy, Al-Saleh, et al., 2012; Talaei et al., 2013). Similarly, confounding factors were, generally not clearly described or discussed. All of the studies discussed their limitations appropriately and interpreted the overall results cautiously.

Table 2.6: Quality assessment of the observational studies included in the systematic review

Authors and year of publication	Study design	STROBE scores
Al-Jabri et al 2010	Cohort	16
Gagnon et al 2012	Cohort	22
T.J Wang et al 2008	Cohort	19
Talaei et al 2008	Cohort	17
Tai et al 2008	Cohort	17
Al-Daghri et al 2012	Cohort	14
AlDgahri et al 2012b	Cohort	19

2.3.5 Frequency, dosage and duration of vitamin D supplementation

Regarding the frequency of vitamin D supplementation, 19 studies provided daily supplementation (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Aljabri et al., 2010; Asemi et al., 2014; Breslavsky et al., 2013; Gepner et al., 2012; Harris et al., 2012; Hsia et al., 2007; Major et al., 2007; K. C. Maki et al., 2011; Mitri et al., 2011; Patel et al., 2010; Pfeifer et al., 2001; Pittas, Harris, et al., 2007; Ryu et al., 2013; Salehpour et al., 2012; von Hurst et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al.,

2009). The vitamin D dosage for daily supplementation ranged from 200 IU to 5,000 IU. Five studies provided weekly vitamin D supplementation; two studies provided 50,000 IU (Asemi et al., 2014; Talaei et al., 2013), one study provided 20,000 IU (Sollid et al., 2014), one study provided 20 drops of Vigantol oil (1904 IU) (Strobel et al., 2014) and one study provided 40,000 IU or 20,000 IU (Jorde et al., 2008)). One study provided only a single dose of vitamin D either 100,000 IU or 200,000 IU (Witham, Dove, et al., 2010). Two studies provided the intervention fortnightly (120,000 IU (Nagpal et al., 2009) and 100,000 IU (Tai et al., 2008)). Two studies did not used vitamin D supplementation as intervention; instead, they provided only advice on vitamin-D rich diet (Tzotzas et al., 2010) or sunlight exposure advice and vitamin D-rich diet advice as the intervention (Al-Daghri, Alkharfy, Al-Saleh, et al., 2012).

The range of intervention duration was between four weeks and seven years. The study duration of 24 studies was one year or less (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Al-Daghri, Alkharfy, Al-Saleh, et al., 2012; Asemi et al., 2014; Breslavsky et al., 2013; Gepner et al., 2012; Harris et al., 2012; Jorde et al., 2008; Major et al., 2007; K. C. Maki et al., 2011; Mitri et al., 2011; Nagpal et al., 2009; Patel et al., 2010; Ryu et al., 2013; Salehpour et al., 2012; Sollid et al., 2014; Strobel et al., 2014; Tai et al., 2008; Talaei et al., 2013; Tzotzas et al., 2010; von Hurst et al., 2010; Witham, Dove, et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009), whereas six studies were found to have longer study duration of more than one year (Al-Daghri, Alkharfy, Al-Othman, et al., 2012; Gagnon et al., 2012; Hsia et al., 2007; Pfeifer et al., 2001; Pittas, Harris, et al., 2007; T. J. Wang et al., 2008).

2.3.6 Serum 25(OH)D assessment

There are many commercially available assays that assess serum 25(OH)D. The most common serum 25(OH)D assays used were competitive protein binding assays (CPBA), radioimmunoassays (RIA), high-pressure liquid chromatography (HPLC), enzyme-

linked immunosorbent assays (ELISA), liquid chromatography tandem mass spectroscopy (LC-MS/MS), and electrochemiluminescent immunoassays (ECLIA). The assays used are summerized in Table 2.7. Out of 30 studies, four (13.3%) studies did not report the laboratory assays used for serum 25(OH)D assessment.

Table 2.7: Distribution of the laboratory assays used for the serum 25(OH)D assessment

Instruments	Frequency (%)
Competitive protein binding assays (CPBA)	3 (10)
Radioimmunoassay (RIA)	12 (40)
High-pressure liquid chromatography (HPLC)	1 (3.3)
Liquid chromatography tandem mass spectroscopy (LC-MS/MS)	2 (6.7)
Elcetro-chemiluminescent immunoassays (ECLIA)	3 (10)
Enzyme-linked immunosorbent assay (ELISA)	5 (16.7)
Not mentioned	4 (13.3)

2.3.7 Outcomes

i. Diabetes mellitus

Out of 25 studies that assessed the effects of vitamin D on diabetes mellitus, only nine studies showed improvement. Among the clinical trials, vitamin D supplementation improved HbA1c in women with type 2 diabetes mellitus, although HOMA was not significant (Strobel et al., 2014); improved 3 h-OGIS in healthy and centrally obese males (Nagpal et al., 2009), decreased HOMA-IR among healthy overweight and obese people (Asemi et al., 2013); improved the disposition index and insulin secretion in overweight individuals with glucose intolerance (Mitri et al., 2011); improved HOMA-IR in osteporotic patients (Pittas, Harris, et al., 2007); improved insulin sensitivity in women of South Asian origin in New Zealand (von Hurst et al., 2010); and improved HOMA-IR in patients with type 2 diabetes mellitus (Al-Daghri,

Alkharfy, Al-Othman, et al., 2012). In the cohort studies, vitamin D was found to improve HOMA-IR in patients with type 2 diabetes mellitus (Talaei et al., 2013) and to improve the HOMA index in obese women (Tzotzas et al., 2010).

ii. Lipid

A total of 17 studies assessed lipid profiles of which only six studies showed significant improvements. In the clinical setting, vitamin D supplementation was effective at reducing TG in healthy overweight and obese individuals (A. Zittermann et al., 2009), reducing TG and VLDL in healthy overweight and obese individuals (Asemi et al., 2013), improving HDL and LDL:ApoB-100 in healthy overweight and obese individuals (Salehpour et al., 2012) and decreasing total:HDL, LDL and LDL:HDL in overweight premenopausal women (Major et al., 2007). In cohort studies, vitamin D improved HDL and TG in a non-diabetic cohort in Saudi Arabia (Al-Daghri, Alkharfy, Al-Saleh, et al., 2012) and improved TG in an Australian cohort without metabolic syndrome (Gagnon et al., 2012).

iii. Blood pressure (BP)

Only 13 studies assessed BP as one of the study outcomes and only three studies showed significant improvement in BP after vitamin D supplementation was provided. There were significant reduction in SBP in type 2 diabetes mellitus patients with low SBP at baseline (Witham, Dove, et al., 2010) elderly women (Pfeifer et al., 2001) and healthy postmenopausal women (Wood et al., 2012).

iv. Metabolic syndrome

Only two studies assessed metabolic syndrome in this review. Both studies showed significant improvement. Vitamin D was found to be effective at reducing the risk of developing metabolic syndrome in an Australian cohort (Gagnon et al., 2012) and non-diabetic subjects in Saudi Arabia (Al-Daghri, Alkharfy, Al-Saleh, et al., 2012).

2.3.8 Conclusion

The overall study quality was high in eight out of 24 RCTs and in all observational studies. All of the included studies improved the vitamin D status of the participants. However, the results were inconsistent in showing that vitamin D was beneficial for improving cardiometabolic risk factors, such as BP, lipid profiles and HOMA-IR. This outcome could be due to the variety of vitamin D dosages and frequencies of consumption, sample sizes, study durations and study populations.

This systematic review also showed that most cardiometabolic risk factors were improved only if the baseline study outcomes were abnormal. Most of the studies used various vitamin D dosage and frequencies, and the majority had study durations of less than one year.

2.4 Systematic review of vitamin D deficiency impact on HRQOL

Bibliographic databases including Embase, PubMed and Cochrane Library (Cochrane Central Register of Controlled Trials), which were limited to English-language articles only, were used in the search. The keywords used were based on the research question, "What is the effect of vitamin D supplements on health-related quality of life in vitamin D-deficient premenopausal women?". However, the terms used in thesearch reflected all age ranges and included males and females. We also included all types of study designs, as there were limited studies on vitamin D status and quality of life in premenopausal women.

The search terms used were as follows: (adults OR (age 35 and above) OR middle aged OR elderly) AND ((vitamin D OR (serum 25(OH)D) AND (quality of life) OR (mental health) OR (physical health) OR (health-related quality of life)). The titles and abstracts of studies conducted before the year 2000 were scanned to identify potential

articles for review. Potentially eligible papers were retrieved in hard copy for more detailed review.

It is well known that vitamin D deficiency has a negative impact on bone health, such as osteoporosis, osteomalacia, muscle weakness, neuromuscular coordination and rickets. These conditions are known to increase the risk of fractures. Many studies have also found associations between vitamin D and mental health issues such as depression (Anglin, Samaan, Walter, & McDonald, 2013; M. B. Humble, 2010; Ju et al., 2013). However, very few studies have investigated the association between vitamin D status and HRQOL. Therefore, this systematic review was aimed at reaching a better understanding of the impact of vitamin D status on HRQOL. A flow chart of the search strategy is shown in Figure 2.6. After the extraction of duplicate articles from the databases, 15 articles were used for this review.

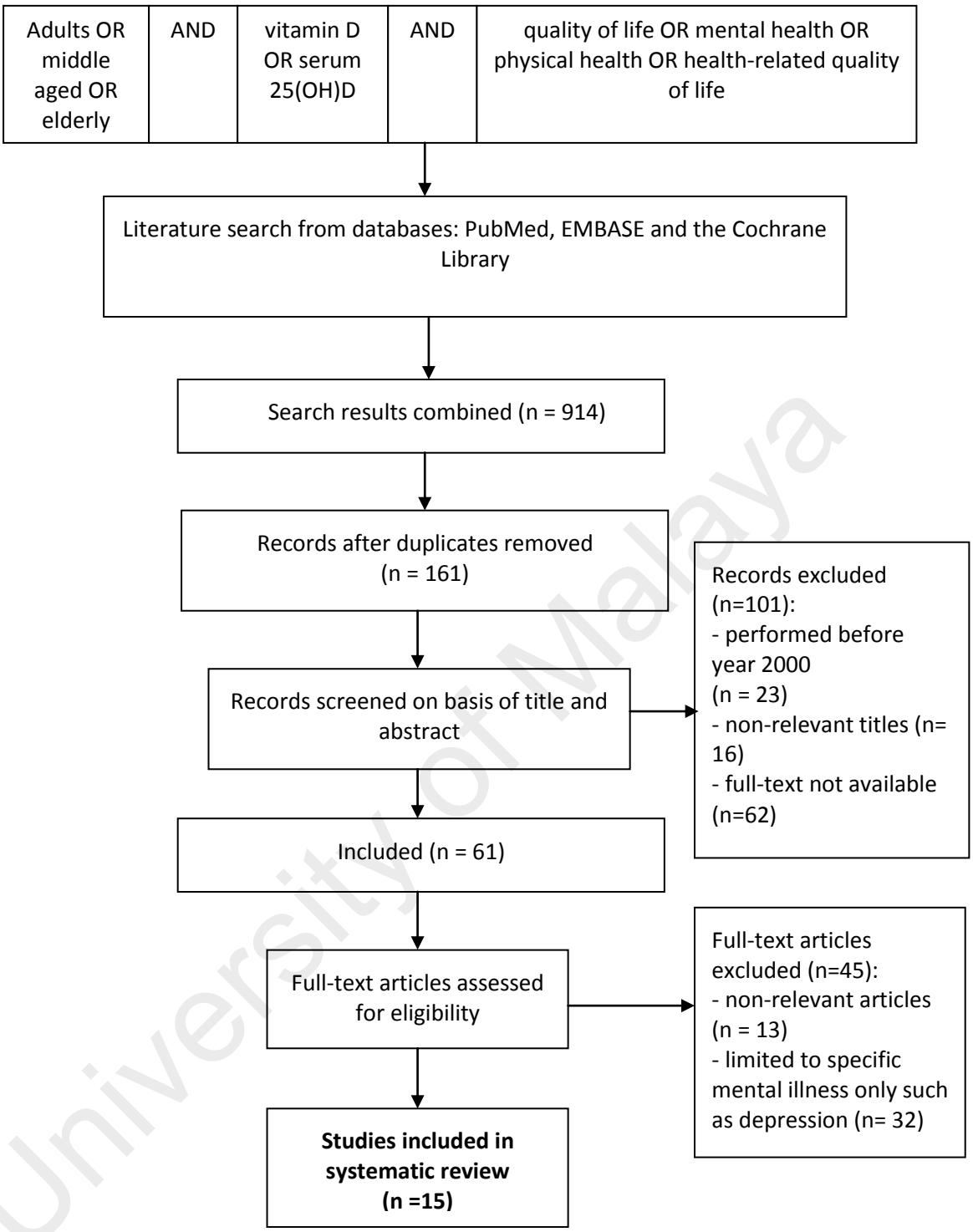

Figure 2.6: Flow chart of search strategy for studies on vitamin D impact on health-related quality of life

The risk of bias of RCTs was assessed using a modified version of the Cochrane Collaboration's tool (J.P.T. & S., 2009). The items included random sequence generation, allocation concealment, blinding of participants, blinding of self-reported outcome assessment, blinding of objective outcome assessment, completeness of outcome data, and selective reporting. For observational studies, the quality of reporting was assessed using the STROBE checklist (von Elm et al., 2008). The studies with reporting quality scores under 13 or with insufficient temporal information between exposure and outcome were considered to be of low quality. The remaining studies were arbitarily categorized into high grade quality scores.

2.4.1 Demographics characteristics

Table 2.8 summarizes the studies selected for the systematic review of the impact of vitamin D deficiency on HRQOL. Fifteen articles were identified, which included five clinical trials (Dean et al., 2011; Jorde et al., 2008; Sakalli et al., 2012; Schreuder et al., 2012; Witham, Crighton, et al., 2010b) and 10 observational studies (Anand et al., 2011; Sibel Basaran et al., 2007; Chao et al., 2014; Ecemis & Atmaca, 2013; W. Huang et al., 2013; Kjaergaard et al., 2012; Le Goaziou et al., 2011; Michael et al., 2011; Motsinger et al., 2012; Ohta, Hamaya, Taketsuna, & Sowa, 2014). The clinical trials were conducted in patients with chronic heart failure, the elderly, and the general community. The observational studies were conducted in healthy premenopausal women, osteoporotic women, patients on dialysis, the elderly, healthy young adults and the general community.

Table 2.8: Effect of vitamin D supplementation in relation to HRQOL

Author	Study design, study population	HRQOL tool used	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Ecemis and Atmaca (2013)	Cross sectional n = 80 Healthy premenopausal women	SF36	Not relevant	Turkey 1st sept to 30th nov 2011	Low PCS, MCS, PF, SF and vitality associated with vitamin D deficient and insufficient women	Vitamin D deficiency: < 50 nmol/l
Sibel Basaran et al. (2007)	Cross sectional n = 259 Osteoporotic women without fractures	VAS QUALEFFO	Not relevant	Turkey April to Sept 2004	Vitamin D deficiency associated with low total QOL including all subscales	Vitamin D deficiency: < 30 nmol/l Some subjects are on vitamin D and Ca treatment
Anand et al. (2011)	Cross sectional n = 192 Subjects in Comprehensive Dialysis Study	SF12 HAP	Not relevant	US June 2005 to January 2007	Low vitamin D concentration associated with lower physical activity and poor mental health	No information on whether participants taking vitamin D supplements
Ohta, Uemura, et al. (2014)	Cross sectional n = 1585 Subjects take part in Japanese Osteoporosis Interventional Trial	JOQOL	Not relevant	Japan	Significantly lower daily living and recreational and social activity domains in low vitamin D group (< 50 nmol/l)	Female only Not taking into account of other confounders (sunlight exposure, diet)

Table 2.8: Effect of vitamin D supplementation in relation to HRQOL (Continued)

Author	Study design, study population	HRQOL tool used	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Witham, Crighton, et al. (2010b)	Double-blind RCT n = 105 aged ≥ 70 years known case of chronic heart failure with vit D < 50 nmol/l	6-minutes walk test Minnesota Living with Heart Failure score	100,000 IU vitamin D2 or placebo at baseline and 10 weeks	Scotland, UK 20 weeks	No improvement in physical function and QOL	Used Vitamin D2 (vitamin D3 have longer half-life)
W. Huang et al. (2013)	Case series n = 28 US veterans with multiple areas of chronic pain and low vit D (< 75 nmol/l)	Veteran Rand 36	50,000 IU of vitamin D3 if serum 25(OH)D < 50 nmol/l or 1200 IU/d of vitamin D if serum 25(OH)D 50 to 74 nmol/l	US 3 months	Improved bodily pain, sleep, GH, vitality and SF Borderline significant for PF and PCS	No comparison group Still continue pain medication during intervention study
Sakalli et al. (2012)	RCT n = 120 Aged > 65 years	SF36 VAS	Single dose of 300,000 IU vitamin D3 orally or IM or oral placebo or IM placebo	Turkey 4 weeks	Single megadose of IM vitamin D improved QOL (PF, RP, BP, GH, SF and MH) and decreased musculoskeletal pain Oral dose improved RP and PF and decreased pain	VAS improved in all group – is it due to Hawthorne effect?

Table 2.8: Effect of vitamin D supplementation in relation to HRQOL (Continued)

Author	Study design, study population	QOL tool used	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Michael et al. (2011)	Longitudinal observational study n = 532 Women aged 50 to 79	Time-walk & chair stand test	Not relevant	US 6 years	Higher baseline serum 25(OH)D was associated with better physical performance	Subjects from WHI CT
Motsinger et al. (2012)	Cross sectional n = 15,954 aged 55 -69 years	5 scales Mental domains from SF36 (MH, RE, SF, Vitality, GH)	Not relevant	Iowa, US 6 years	Vitamin D < 400 IU/d had significantly lower mental health HRQOL	Part of Iowa Women's Health study
Chao et al. (2014)	Cross sectional n = 1,493 Aged > 50 years	EQ-5D-5L	Not relevant	Alberta, Canada	An increased of 100 nmol/l serum 25(OH)D were associated with increased of 29% in HRQOL Vitamin D associated with mobility, usual activities, depression and anxiety	Not measured sunlight exposure history and diet as confounder factors
Jorde et al. (2008)	RCT n = Overweight and obese	BDI	20,000 IU/week or 40,000 IU/week vitamin D or placebo	Norway 1 year	Low vitamin D level associated with depression symptoms High dose vitamin D improved depression symptoms	Physical activity was measured using IPAQ

Table 2.8: Effect of vitamin D supplementation in relation to HRQOL (Continued)

Author	Study design, study population	QOL tool used	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Schreuder et al. (2012)	Semi-crossover RCT n = 84 Aged 18 – 60 years	VAS	Single dose Vitamin D 150,000 IU or placebo At week 6: second randomization for intervention group to received placebo or active intervention All placebo switched to vitamin D group	Netherlands 12 weeks	Small positive effect on pain after high dose of vitamin D	Serum PTH, diet history, sunlight exposure history not measured
Kjaergaard et al. (2012)	Nested case control: n = 357 RCT: n = 243 Aged 30 – 75 years	BDI Hospital anxiety and depression scale Seasonal pattern assessment scale Montgomery-Asberg depression rating scale	20,000 IU/week vitamin D or placebo	Norway 6 months	Low level vitamin D associated with depression but no effect was found with vitamin D supplements	Part of Tromso Cohort study
Dean et al. (2011)	Double-blind RCT n = 128 Healthy young adults No psychiatric illness or cognitive dysfunction	BDI	5000 IU/d vitamin D or placebo	Queensland, Australia 6 weeks	Vitamin D not influenced cognitive or emotional functioning in healthy young adults	Healthy population Wide range of vitamin D status in study population

Table 2.8: Effect of vitamin D supplementation in relation to HRQOL (Continued)

Author	Study design, study population	QOL tool used	Intervention / exposure comparison	Location & Study duration	Main findings	Comments
Le Goaziou et al. (2011)	Cross sectional study n = 196 vitamin D deficiency women aged 20 to 50 years	SF12	Not relevant	Rhoe Alps, France	Participants with vitamin status < 30 nmol/l had low PCS and PF	Vitamin D deficiency premenopausal women

2.4.2 Types of intervention

One study used a single dose of 300,000 IU of vitamin D3 orally or intra-muscularly (Sakalli et al., 2012), one study used 100,000 IU of vitamin D2 at baseline and at 10 weeks (Witham, Crighton, et al., 2010b), one study used 20,000 IU/week or 40,000 IU/week of vitamin D (Jorde et al., 2008), and one study used 5000 IU of vitamin D daily (Dean et al., 2011). A semi-cross over RCT used a single dose of 150,000 IU of vitamin D as their intervention (Schreuder et al., 2012). All five RCTs compared the intervention with placebo.

2.4.3 Study designs

There were various study designs used to assessed the impact of vitamin D deficiency on HRQOL. In this systematic review, five studies were RCTs with one of them having a semi-cross over trial design (Dean et al., 2011; Jorde et al., 2008; Sakalli et al., 2012; Schreuder et al., 2012; Witham, Crighton, et al., 2010a). Out of 10 observational studies, seven were cross-sectional studies (Anand et al., 2011; Sibel Basaran et al., 2007; Chao et al., 2014; Ecemis & Atmaca, 2013; Le Goaziou et al., 2011; Motsinger et al., 2012; Ohta, Uemura, et al., 2014) and one each were case series (W. Huang et al., 2013), case control (Kjaergaard et al., 2012), and cohort studies (Michael et al., 2011).

2.4.4 HRQOL instrument

A variety of HRQOL instruments were used to assess the physical and mental health of individual with vitamin D deficiency. The instruments used for HRQOL assessments are shown in Table 2.9. Two studies used both HRQOL instruments with a visual analogue scale for pain (Sibel Basaran et al., 2007; Sakalli et al., 2012).

Table 2.9: Distribution of the instruments used for HRQOL assessment

Instruments	Frequency (%)
SF36	3 (20)
SF12	2 (13.3)
Quality of Life Questionnaire of the European Foundation for Osteoporosis (QUALEFFO)	1 (6.7)
Japanese Osteoporosis Quality of Life (JOQOL)	1 (6.7)
Minnesota living with heart failure	1 (6.7)
Veteran Rand 36 (modified SF36)	1 (6.7)
EQ-5D-5L	1 (6.7)
Beck Depression Inventory (BDI)	3 (20)
Time walk and chair stand test (physical health only)	1 (6.7)
Visual analogue scale only	1 (6.7)

2.4.5 Quality assessment

The risk of bias quality assessment for vitamin D supplementation on HRQOL using the Cochrane risk of bias tool in the included studies is summarized in Figures 2.7 and 2.8. Random sequence generation, allocation concealment, blinding of participants and outcome assessment were adequately reported in all of the trials, except for two (Jorde et al., 2008; Sakalli et al., 2012) and were sufficient in all of the protocols. Selective reporting bias was detected in only one study (Jorde et al., 2010). None of the study protocols were funded by pharmaceutical companies. Nevertheless, funding sources were not reported in the disclosures of two study protocols (Sakalli et al., 2012; Schreuder et al., 2012), thus providing insufficient information to permit judgement.

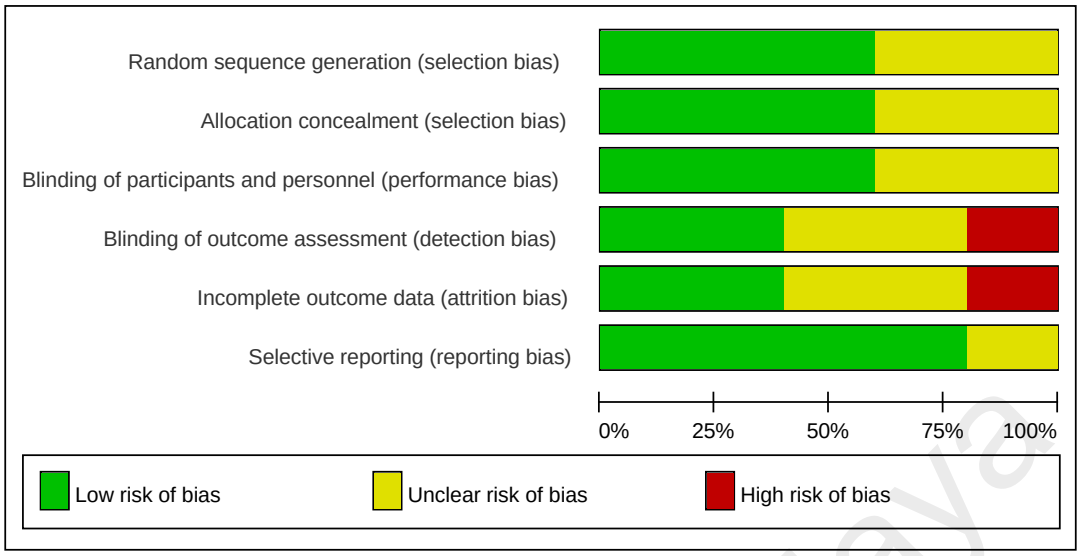

Figure 2.7: Risk of bias graph reviewing the judgements about each risk of bias item, which are presented as percentages across all included studies

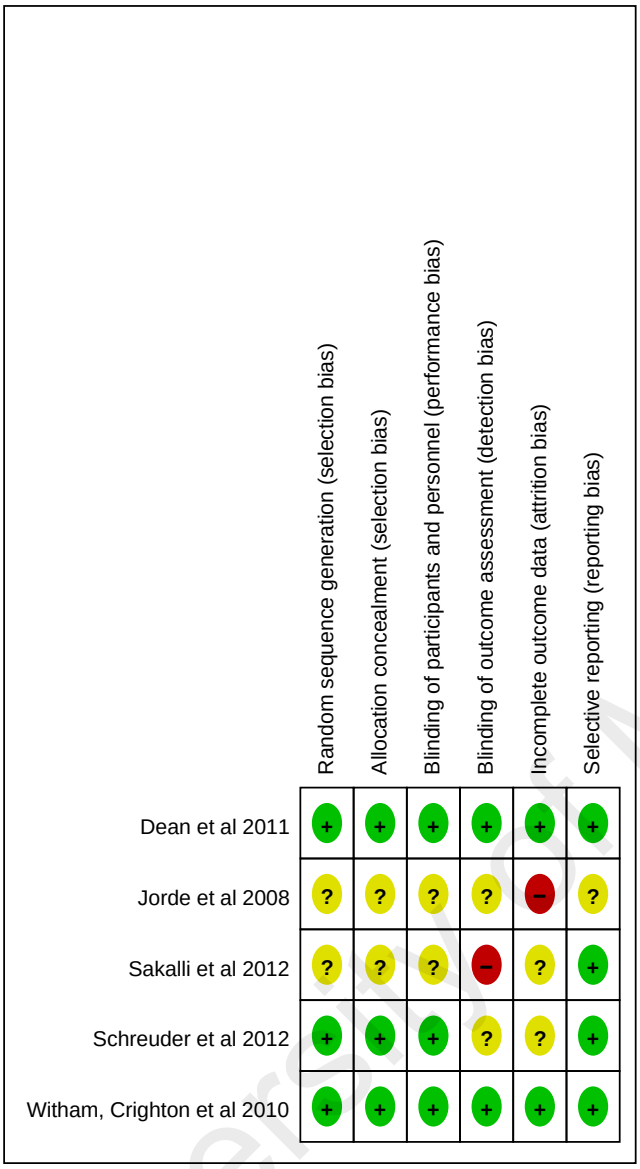

Figure 2.8: Risk of bias summary which review the judgements about each risk of bias item for each included study.

Table 2.10 summerizes the results of the quality of reporting using the STROBE checklist for vitamin D supplementation on HRQOL. Items in the title and abstract, introduction and methodology were reported sufficiently in all of the studies. However, sample size justification by describing sample size calculations was detected in only one study (Kjaergaard et al., 2012).

Only five studies (Sibel Basaran et al., 2007; Kjaergaard et al., 2012; Michael et al., 2011; Motsinger et al., 2012; Ohta, Hamaya, et al., 2014) had adequate reporting of non-participation at each stage and used flow diagrams to represent numbers of participants; most of the studies were inadequate in these areas. Nevertheless, none of the studies included in this systematic review described how missing data were handled.

All of the selected papers provided a clear account of the outcomes, exposures, effect modifiers and confounders being assessed. However, low reporting of statistical methods to address confounding was observed; this factor was adequate in only seven studies (Anand et al., 2011; Sibel Basaran et al., 2007; Chao et al., 2014; Le Goaziou et al., 2011; Michael et al., 2011; Motsinger et al., 2012; Ohta, Hamaya, et al., 2014). Nevertheless, confounding factors were, on the whole, not clearly described or discussed. The items in the discussion were appropriately addressed by all of the studies.

Table 2.10: The quality assessment of studies included in the systematic review

Authors and year of publication	Study design	STROBE scores
Ecemis and Atmaca 2013	Cross-sectional	17
Sibel Basaran et al 2007	Cross-sectional	19
Anand et al 2011	Cross-sectional	19
Ohta et al 2014	Cross-sectional	8
Huang et al 2013	Cross-sectional	19
Michael et al 2011	Case series	20
Motisnger et al 2012	Cohort	19
Chao et al 2014	Cross-sectional	18
Kjaergaard et al 2012	Cross-sectional	21
Le Goaziou et al 2011	Cross-sectional	21

2.4.6 Outcomes

Nine studies assessed both components of HRQOL which are the physical and mental health components. Eight out of nine studies showed a significant association between vitamin D status and HRQOL. Vitamin D status was found to be positively associated with HRQOL in healthy premenopausal women (Ecemis & Atmaca, 2013), osteoporotic women without fractures (Sibel Basaran et al., 2007; Ohta, Uemura, et al., 2014), patients on dialysis (Anand et al., 2011), elderly in the community (Chao et al., 2014; W. Huang et al., 2013; Sakalli et al., 2012) and subjects in the community aged 20 to 50 years (Le Goaziou et al., 2011). Only Witham, Crighton, et al. (2010b) found no improvement in physical function or QOL in elderly patients with chronic heart failure. In assessing pain improvement with vitamin D supplementation, three studies found that vitamin D supplementation had some positive effects on pain (W. Huang et al., 2013; Sakalli et al., 2012; Schreuder et al., 2012). Only one study assessed the association between vitamin D status and physical health; it found that higher baseline serum 25(OH)D was associated with better physical performance (Michael et al., 2011). For mental health and depression, inconsistent results were found in four of the studies included in this review. Vitamin D supplementation did not influence cognitive or emotional functioning in healthy young adults (Dean et al., 2011). Although Kjaergaard et al. (2012) found that low levels of vitamin D were associated with depression, no effect of vitamin D supplementation was found. In contrast, Jorde et al. (2008) observed that high dose of vitamin D did improve depression symptoms in overweight and obese individuals. A study by Motsinger et al. (2012) found that individuals with low intake of vitamin D (less than 400 IU per day) had significantly lower mental HRQOL.

2.4.7 Conclusion

The overall study quality was high in two out of five RCTs and nine out of ten observational studies. This systematic review showed that a considerable number of published observational studies found an association between vitamin D status and HRQOL. However, inconsistent results were found in clinical trials. Most of these studies were performed in elderly subjects with medical conditions, such as chronic heart failure or osteoporosis, who were at higher risk of vitamin D deficiency. Except for a clinical trial conducted in elderly subjects with known chronic heart failure, all of the other studies showed that vitamin D was beneficial in improving HRQOL. This review also showed that low HRQOL was associated with vitamin D deficiency in young, healthy adults. Nevertheless, the true impact of vitamin D supplementation on HRQOL remains unclear because limited clinical trials were available. Therefore, more clinical trials are needed to clarify this issue.

2.5 Common statistical methods used to analyze repeated measures data

There are a variety of statistical methods used for analyzing repeated continuous outcome measures data. These studies collect data on outcome measures from the same subject at multiple time points, which provides an opportunity to examine how the outcome measures change over time in both the treatment effect and the subject.

2.5.1 Repeated measures analysis of variance (ANOVA)

The most common methods of statistical analysis for repeated measures studies include the conventional repeated measures ANOVA. Repeated measures ANOVA is a test that measures whether there are any differences between related population means to determine whether the populations' means are equal (null hypothesis). However, repeated measures ANOVA does not address where the differences between groups lie. Instead, if a repeated

measures ANOVA result is found to be statistically significant, a post hoc test must be used to highlight where the differences occur.

A repeated measures ANOVA formula to calculate an F-statistic is as below:

$$F = \frac{MS_{time}}{MS_{error}}$$

To determine the mean sum of squares for time, MS_{time}, the following calculation is used:

$$MS_{time} = \frac{SS_{time}}{(k-1)}$$

where k = the number of time points and $(k-1)$ = the degree of freedom.

To determine the mean of sum of squares for error, MS_{error}, the sum of squares for error is divided by $(n-1)(k-1)$ degrees of freedom. n = the number of subjects and k = the number of time points. The formula for the calculation is shown below:

$$MS_{error} = \frac{SS_{error}}{(n-1)(k-1)}$$

The advantage of using repeated measures ANOVA is that it can further partition the error variability (SS_{error}), reducing its size; thus, it has the effect of increasing the value of the *F*-statistic due to the reduction of the denominator. This effect leads to an increase in the power of the test to detect significant differences between means, whereas in an independent (between-subjects) ANOVA, within-group variability (SS_w) expresses the error variability (SS_{error}).

There are several significant problems with repeated measures ANOVA, which are the assumption of sphericity and the requirement for complete data sets. Repeated measures ANOVA is particularly susceptible to violations of the assumption of sphericity. To

overcome the problem of the violation of the sphericity assumption in repeated measures ANOVA, the lower-bound estimate**,** Greenhouse-Geisser and Huynh-Feldt corrections are used. However, these corrections could contribute to loss of power because they may fail to detect an effect on the overall p-value or the subsequent test. In repeated measures ANOVA, missing data are not allowed; hence, it requires complete data sets for analysis. For example, if one observation or measurement of a subject is missed due to various reasons, then all of the data from that subject must be discarded. This limitation affects the power of the trial and consequently reduces precision in estimating group comparisons, possibly leading to biased results. For decades, a common method of handling missing data was to replace the missing observation with last observation carried forward (LOCF) or with a subject's mean approach. These strategies, however, may result in underestimation of the error variance. In addition, they give the researcher a false sense of security about the need to resolve complex issues of non-adherence, which has led investigators to under-design studies in the mistaken belief that LOCF will cure all (Lavori, Brown, Duan, Gibbons, & Greenhouse, 2008).

2.5.2 Linear mixed effects (LME) models

An LME model is a statistical model containing both fixed effects and random effects. Similar to repeated measures ANOVA, an LME model is used when repeated measurements are made on the same statistical units or where measurements are made on clusters of related statistical units. In an LME model, fixed and random effects are thought to be the sum (linear). If an effect, such as a medical treatment, affects the population mean, it considered is a fixed effect. On the other hand, if the effect is associated with a sampling procedure, then it is a random effect.

A linear mixed effects model can be represented by the following equation:

$$y = X\beta + Zu + \epsilon$$

where y = known vector of observations, with mean $E(y) = X\beta$; β = unknown vector of fixed effects; u = unknown vector of random effects, with mean $E(u) = 0$ and variance-covariance matrix $var(u) = G$; ϵ = unknown vector of random errors, with mean $E(\epsilon) = 0$ and variance $var(\epsilon) = R$; and X and Z are design matrices relating observation y to β and u, respectively.

An LME model is based on maximum likelihood (ML) and restricted maximum likelihood (REML) methods. This method yields asymptomatically efficient estimators for balanced and unbalanced designs. Compared to the conventional repeated measures ANOVA, this method produces an optimum estimator (minimum variance) for balanced designs only. Because data are usually unbalanced due to missing data, linear mixed effects models present an advantage over ANOVA methods.

LME models provide a parsimonious way to represent the group mean trajectory and covariance structure between serial measurements; they are able to handle data with missing data in any variable across time points and imperfect timing; and they make use of subjects with a single time point to characterize inter-subject variation (Dijkers, 2013). Through this approach, all subjects with missing data may be included in the analysis. Therefore, unlike repeated measures ANOVA, an LME analysis has increased statistical power because it includes all of the data from all of the subjects.

Compared to repeated measures ANOVA, LME models offer a more powerful and versatile framework for a repeated continuous outcome measures analysis. LME is robust to violations of sphericity assumptions, homoscedasticity and missing data.

Another advantage of LME models is that follow-up times do not have to be measured at the same time for all of the subjects because time is treated as continuous variable. In some longitudinal studies, follow-up times are not uniform across all of the subjects. In LME models, both time-varying and time-invariant covariates are included. Therefore, any changes in the outcome variable could be due to a subject's fixed characteristics, such as gender, or any characteristics that change across time, such as life events (Gibbons, Hedeker, & DuToit, 2010).

In LME, interactions between individual characteristics and the treatment can be analyzed using the covariates in fixed-effects models. Additionally, the covariates for the analysis may be nominal or numeric. Repeated measures ANOVA is also able to analyze the effects of covariates on the treatment; however, unlike LME models, it can only provide a p-value for the interaction and not the magnitude of the effect, which is much more important.

CHAPTER 3 : METHODOLOGY

3.1 Study design

This was a double-blind, randomized, placebo-controlled, parallel trial in which the participants were randomized at a 1:1 ratio.

3.2 Study area

This study was conducted at the University of Malaya, Kuala Lumpur, Malaysia. Malaysia is a tropical country with almost year-round sufficient UVB radiation wavelength necessary for the cutaneous synthesis of vitamin D. Kuala Lumpur, the capital city of Malaysia, is located near the equator at a latitude of 03° 09' north. The University of Malaya is situated on a 922-acre campus in south western Kuala Lumpur. This multi-disciplinary research university has 17 faculties and research centres. According to statistics, there were 2856 academic staff in 2013, and the non-academic staff consisted of management and professionals (768 staff) and support staff (3064 staff) (University of Malaya, 2013).

3.3 Study population

The study population was premenopausal female employees of the University of Malaya aged between 30 and 55 years. The female employees who work at this university range from academic staff, such as professors and lecturers, to non-academic professional staff, such as managers, nurses, and secretaries. The non-academic support staff consists of clerks and general workers, such as drivers, office cleaners, electricians and gardeners.

3.4 Sample size estimation

To detect reductions in cardiometabolic risk factors, we calculated the sample size based on a study by Zitterman et al (2009). The results of this study indicate that vitamin D

supplementation was able to significantly improve only triglycerides (a reduction of 0.19 ± 0.54 mmol/L in the intervention group, whereas there was an increase of 0.03 ± 0.50 in the placebo group) and TNF-α (a reduction of 0.80 ± 2.5 in the intervention group, whereas there was an increase of -0.26 ± 2.9 in the placebo group) in vitamin D deficient subjects. However, compared with placebo, vitamin D supplementation resulted in an increase in LDL. Therefore, the triglyceride outcomes from this study were used to calculate the sample size. To detect a reduction in cardiometabolic risk factors with a two-sided 5% significance level and a power of 80%, a minimum of 88 subjects were required for each arm. Assuming a drop-out rate of 10%, 97 subjects were recruited per arm. The sample size calculation was conducted using OpenEpi Software version 2.3.1.

3.5 Definition of terms

i. Vitamin D deficiency

For this study, we used Holick's (2011) definition of vitamin D deficiency which was defined as a serum 25(OH)D level of less than 50 nmol/l (20 ng/ml). Vitamin D insufficiency was defined as 51 to 74 nmol/l (21 to 29 ng/ml) and vitamin D levels ≥ 75 nmol/l (30 ng/ml) were considered normal.

ii. Cardiometabolic risk

The term "cardiometabolic risk" refers to a situation in which the possibility of developing atherosclerotic cardiovascular disease and diabetes mellitus is significantly enhanced due to the presence of insulin resistance, high blood pressure, high blood glucose, low HDL, high triglyceride levels and high LDL (Brunzell et al., 2008).

iii. High blood pressure

High blood pressure was defined as systolic blood pressure (SBP) ≥ 130 mmHg and diastolic blood pressure (DBP) ≥ 85 mmHg.

iv. Abnormal lipid profile

Abnormal lipid profile was defined as TG level ≥ 1.7 mmol/l, HDL ≤ 1.29 mmol/l or LDL ≥ 2.6 mmol/l.

v. High insulin resistance

Insulin resistance was evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR) and was calculated as fasting glucose (mmol/L) x insulin (mIU/mL) / 22.5 (Matthews et al., 1985). Abnormal insulin resistance for the Malay population was defined as a HOMA-IR level > 1.05 (A. K. Al-Mahmood, A. A. S. Ismail, F. A. Rashid, & W. M. Wan Bebakar, 2006).

vi. Metabolic syndrome

According to the harmonized criteria of metabolic syndrome, metabolic syndrome was defined as the presence of three or more metabolic risk factors in one person. These risk factors are central obesity, which is measured by waist circumference (female ≥80 cm); fasting blood TG ≥1.7 mmol/l or the use of medication for high TG; low HDL level (less than 1.3 mmol/l in females) or the use of medication for low HDL; elevated blood pressure (SBP ≥ 130 mmHg and/or DBP ≥85 mmHg) or the use of antihypertensive medication; and fasting blood sugar of 5.6 mmol/l or higher or the use of medication for diabetes mellitus (Alberti et al., 2009).

vii. Body mass index (BMI)

The definition of normal, overweight and obese for Malaysians of BMI = 18.5 to 22.9 kg/m^2, 23.0 to 27.4 kg/ m^2 and ≥ 27.5 kg/ m^2, respectively, were used (Ismail IS, 2004).

3.6 Eligibility criteria for participants

3.6.1 Inclusion criteria

i. Vitamin D deficient: serum 25(OH)D level ≤ 50 nmol/l (20 ng/ml).

ii. Premenopausal women: women who did not fulfil the criteria for menopause (WHO, 1996).

    Menopause is defined as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity, and it is recognized to have occurred after 12 consecutive months of amenorrhea for which there is no other obvious pathological or physiological cause can be identified. Menopausal women were excluded from this trial because it would not be ethical to give them a placebo as they were known to have a higher risk of osteoporosis and fracture due to their physiological condition. Menopausal women tend to have 3% to 5% reductions in bone mass due to the loss of oestrogen stimulation on the skeleton. Therefore, they need vitamin D to increase calcium absorption in the gastrointestinal tract to reduce the risk of osteoporosis, osteopenia and fracture (H. Bischoff-Ferrari, 2009; Whitmore, 1996; WHO, 1996).

iii. University of Malaya employees aged 30 to 55 years old at the time of screening.

3.6.2 Exclusion criteria

i. Participants taking vitamin D supplements containing more than 1000 IU/day of vitamin D or any form of calcitriol (1,25(OH)D).

ii. Participants with serum calcium levels greater than 2.7 mmol/l (10.4 mg/dl) at baseline and six-month follow-up.

Elevated serum calciumwas used as an indication of vitamin D intoxication, and participants found to have hypercalcaemia were excluded from this trial. Vitamin D intoxication was defined as a serum 25(OH)D level greater than 375 nmol/l (150 ng/ml), which is associated with symptoms of hypercalcaemia, hypercalciuria and often hyperphosphataemi (Holick, 2009).

iii. Participants with a serum PTH level greater than 7.3 pmol/l (55 pg/ml) at baseline.

Participants who were found to have an abnormal serum PTH level during screening were also excluded from the study to avoid the inclusion of participants with secondary hyperparathyroidism. Secondary hyperparathyroidism plays significant role in the pathogenesis of age-related bone loss and is a risk factor for fractures (Laufey Steingrimsdottir, 2005).

iv. Participants known to have chronic illnesses, such as primary hyperparathyroidism, granuloma-forming disorders (e.g., tuberculosis), lymphoma, sarcoidosis or any type of cancer, were also excluded. Patients with granuloma-forming disorders frequently have hypercalcaemia secondary to elevated serum 1,25(OH)D levels. Thus, vitamin D supplementation may worsen these patients' conditions.

v. Participants who were pregnant.

It is now evident that pregnant women are at very high risk of vitamin D deficiency. Vitamin D deficiency is known to increase the risk of pre-eclampsia by three- to four-fold (Bodnar et al., 2007). Therefore, participants who were pregnant at some point in the trial were excluded from this study due to ethical considerations.

3.7 Conduct of the study

The study was divided into two phases: phase one and phase two. Figure 3.1 illustrates the recruitment process from screening through the end of the intervention.

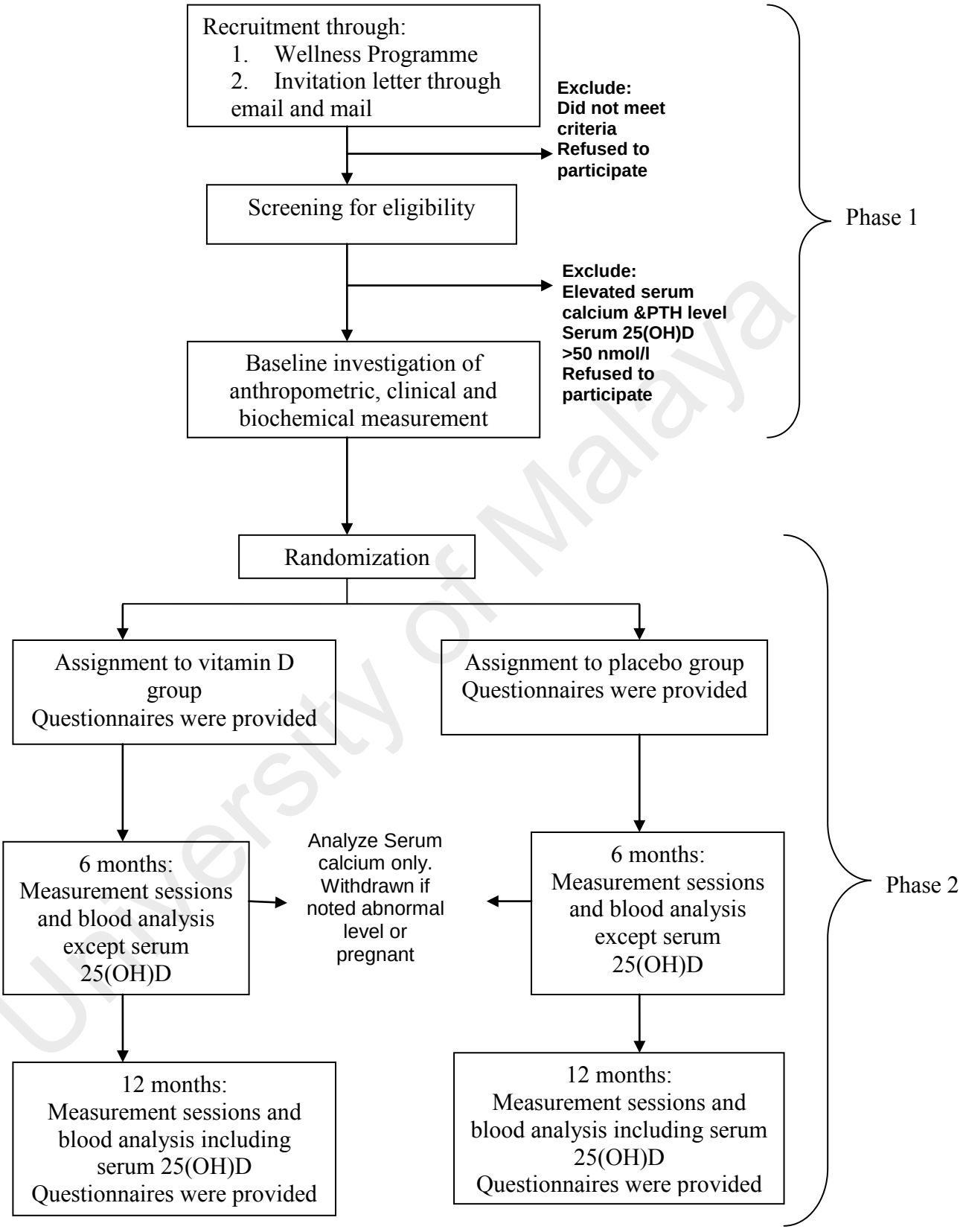

Figure 3.1: Recruitment flow diagram

3.7.1 Phase one

Recruitment and screening were conducted during phase one. The initial screening occurred during the university's wellness programme from May 2012 to July 2012. The University of Malaya Wellness Programme is a yearly health screening programme for University of Malaya employees. All employees aged 30 years and above were invited to participate in a free health screening conducted by the Department of Social and Preventive Medicine, Faculty of Medicine, University of Malaya. At first, all of the premenopausal women attending the wellness programme were invited to participate in this trial. The participants were given a brief introduction regarding the trial at this stage. They were informed of the possibility of being excluded from the trial if their blood analysis results did not fulfil the eligibility criteria of the trial and the possibility of being assigned to the placebo group if they were eligible for the trial. In addition to the Wellness Programme's routine screening measurements of BMI, BP, full blood sugar (FBS) and lipid profiles, the participants were offered blood analyses of serum 25(OH)D, serum calcium, fasting blood insulin and serum PTH as part of the trial eligibility screening. In addition to the recruitment of participants during the university's wellness programme, invitation letters through email and mail were also sent to invite female employees to participate in this study. Individuals who were interested in participating were given an appointment for anthropometric, clinical and biochemical measurements. At this phase, informed consent was obtained from all of the participants for blood sample collection. Personal details and contact information were also obtained from the participants for the purpose of disseminating the results.

Participants who met the inclusion criteria were contacted via telephone or mail. They were briefed regarding their blood results and were invited to participate in the trial.

Another detailed briefing was provided to participants in small groups. Written informed consent (Appendix A) was obtained, and a patient information sheet (Appendix B) was provided. Participants who did not fulfil the inclusion criteria received their blood results via mail or email. Advice on supplementation or medical referrals was provided in the same manner.

3.7.2 Phase two

The randomization and intervention of the first cohort (*n=110)* commenced in October 2012 and ended in September 2013, whereas the second cohort (*n=82)* commenced in January 2013 and ended in December 2013. Participants who consented to this trial were required to complete the following questionnaires at baseline: a background questionnaire (Appendix C), the SF-36 health survey (Appendix D), a sunlight exposure questionnaire (Appendix E) and the International Physical Activity Questionnaire (Appendix F). Details are provided in section 3.11.

Throughout the trial, participants were advised to maintain their usual diet and daily physical activity. They were also told to avoid any vitamin supplements that contained vitamin D or any other supplements other than those they were taking before the trial. All of the participants were given a specially designed booklet (Appendix G) containing appointment dates for intervention sessions and measurement sessions. The participants were asked to keep the scheduled appointments and to contact the researcher if they needed to reschedule an appointment. The booklet also detailed the signs and symptoms of vitamin D intoxication, and participants were advised to seek immediate medical treatment if they had any of these signs and symptoms. The participants were also advised to contact the researchers immediately if they suspected a reaction to the supplements.

The participants returned for measurements after six months and 12 months. If clinical conditions requiring attention were discovered (uncontrolled diabetes or hypertension) during the six-month follow-up, the participants were advised to contact their own physician for treatment with a referral letter from the researcher. All treatments during follow-up, including medications, were recorded.

At 12 months, in addition to taking measurements, all of the participants were requested to complete the questionnaires again, except the background questionnaire. In addition, to ensure compliance with the intervention, the participants were required to consume the active supplement or placebo in front of the researcher at every follow-up visit. For participants who were unable to attend the appointment, the researcher visited them at their workplace to personally give them the active supplement or placebo. If the researcher was unable to personally observe the participant consuming the active supplement or placebo, a reminder to comply with the intervention was provided via telephone, text message or email. During the fasting month of Ramadan, Muslim participants were allowed to consume the active supplement or placebo at home after breaking fast, and similar reminders were also provided. The participants and researchers were blinded to all of the biochemical measurement results at six and 12 months.

3.8 Reports of adverse reaction

All of the participants were given a booklet containing information about the signs and symptoms of vitamin D intoxication. Vitamin D intoxication was defined as a serum 25(OH)D level greater than 375 nmol/l (150 ng/ml), which is associated with symptoms of hypercalcaemia, hypercalciuria and often hyperphosphataemia. The participants were advised to seek immediate medical treatment if they had any of these signs and symptoms.

The participants were also advised to contact the researchers immediately if they suspected an adverse reaction to the supplements.

In addition, at 6 months, the serum calcium levels of all of the participants were analyzed for hypercalcaemia by the researcher. Elevated serum calcium was used as an indication of vitamin D intoxication. Participants found to have hypercalcaemia were excluded from this trial and were referred to an endocrinologist for further investigation and treatment.

3.9 Intervention

All of the participants were given appointment dates in the specially designed booklet. They were required to take their active supplement or placebo once per week for eight weeks, followed by once per month for 10 months. The active supplement and placebo were pre-packed in identical bottles labelled only with the participant's name. The participants were supposed to consume the active supplement or placebo in front of the researcher if possible.

3.9.1 Intervention group

All of the participants in the intervention arm were prescribed the following vitamin D3 (cholecalciferol) supplements: 50,000 IU once per week for eight weeks, followed by 50,000 IU once per month for 10 months for maintenance. The 50,000 IU of dry cholecalciferol had an average weight of 0.5 gram in the form of off-white to yellowish free-flowing particles. This supplement could be consumed at any time of the day, either before or after a meal, and was taken orally by diluting the dry vitamin D in warm water or juice before consumption. The vitamin D supplement was purchased from DSM Nutritional

Products Ltd (Switzerland) and was pre-packed in identical bottles with no identification labels by the Pharmacy Department of the University of Malaya Medical Centre (UMMC).

3.9.2 Placebo group

The placebo was identical to the active supplement in terms of taste, colour and external appearance, but it had no detectable trace of vitamin D. Starch was used as the placebo to match the appearance and taste of the dry vitamin D3. The placebo was pre-packed in bottles without identification labels that were identical to the active supplement bottles, prepared by the researcher and checked by the Pharmacy Department of UMMC to ensure that it contained no trace of vitamin D. The placebo was provided to the participants in the control arm in a manner similar to the intervention arm.

3.10 Randomization procedures

3.10.1 Sequence generation and randomization

The randomization sequence was created using GraphPad software with a 1:1 allocation by a staff member (AC) with no involvement in the trial.

3.10.2 Allocation concealment

The list of eligible participants was matched with the list of generated random numbers. The final list of allocation was kept by AC in a sealed envelope in a locked drawer inaccessible to the researchers. The allocation sequence was fully concealed from the researcher who enrolled and assessed the participants. The allocation list was opened by AC only when it was time to allocate the active supplement and placebo. All of the pre-packed bottles containing the active supplement and placebo were provided to AC for labelling the participants' names one day before each appointment session. The pre-packed

bottles were labelled by AC with the participant's name only according to the allocation list.

3.10.3 Blinding

After receiving the bottles containing the active supplement and placebo from AC, the researcher sent the pre-packed bottles to the respective participants according to the names on the bottle labels. The contents were not listed on the bottle labels to maintain the allocation concealment. The participants were kept blinded to the allocation until the end of the trial. In addition, the researcher, staff and participants were kept blinded to outcome measurements and trial results until the end of the trial unless there were any abnormalities in the serum calcium results indicating vitamin D intoxication.

3.11 Study outcome measures

The study outcome measures and the times of measurement are shown in Table 3.1.

Table 3.1: Measurements obtained during the study period

Measurements for the intervention and placebo groups	Months		
	0	6	12
Biochemical analysis			
25(OH)D	x	x	x
Calcium	x	x	x
PTH	x	x	x
Blood glucose	x	x	x
Blood insulin	x	x	x
HOMA-IR	x	x	x
LDL	x	x	x
HDL	x	x	x
TG	x	x	x
Anthropometric measurements			
Height	x	x	x
Weight	x	x	x
BMI	x	x	x
Waist circumference	x	x	x
Clinical measurement			
Blood pressure	x	x	x
Self-administered questionnaires			
7-days diet diary	x		x
SF-36v2	x		x
Sunlight exposure	x		x
IPAQ	x		x
Socio-demographic & medical history	x		

3.11.1 Primary outcomes

The primary outcomes were cardiometabolic risk factors, such as TG, HDL, LDL, HOMA-IR and BP, serum 25(OH)D and HRQOL. The participants' TG, HDL, LDL, HOMA-IR and BP and serum 25(OH)D were measured at baseline and at six and 12 months. HRQOL was assessed at baseline and at 12 months. Fasting venous blood was

withdrawn for measurements of serum 25(OH)D, TG, HDL, LDL, blood glucose and blood insulin. The outcome measures in this trial were as follows:

i. Serum 25(OH)D:

Any significant increase in serum 25(OH)D from baseline was considered an improvement in the serum 25(OH)D level.

ii. Cardiometabolic risk factors:

a. Insulin resistance:

Any significant reduction in HOMA-IR from baseline was considered an improvement in insulin resistance.

b. Lipid profile:

Any significant increase in the HDL level or reduction in the TG or LDL level from baseline was considered an improvement in the lipid profile.

c. Blood pressure:

Any significant reduction in systolic and diastolic blood pressure from baseline was considered an improvement in blood pressure.

iii. HRQOL:

Any significant increase from baseline in HRQOL components, such as physical functioning, physical role functioning, bodily pain, general health, vitality, social functioning, emotional role functioning, mental health, PCS or MCS, was considered an improvement in HRQOL.

3.12 Data collection

Data collection was performed before the intervention (baseline) and six and 12 months after the commencement of the intervention.

3.12.1 Field work strategy

The participants' baseline data and measurements were collected over a period of three months from May 2012 to July 2012. Subsequently, data were collected six and 12 months after the commencement of the intervention.

All of the participants underwent anthropometric, BP and biochemical measurements at each data collection session. Diet diaries and IPAQ and SF36v2 questionnaires were distributed only at baseline and after 12 months of the intervention.

3.12.2 Blood sampling and biochemical analysis

Venous blood samples were collected by a registered staff nurse between 7.30 am and 9.30 am. During the data collection period, all of the participants were asked to fast overnight for at least eight hours before blood collection. Blood serum was used for the analysis of calcium, PTH, insulin, 25(OH)D and lipid profiles. Blood plasma was used to analyze fasting blood glucose. All of the samples were sent to the Clinical Diagnostic Laboratory (CDL) of the University Malaya Medical Centre (UMMC) for analysis within two hours of blood collection, except for samples for serum 25(OH)D. These samples were kept at room temperature for 30 to 60 minutes to allow for clotting; then, they were centrifuged at 3500 revolutions per minute for 15 minutes. The serum was separated and stored at -80°C until analysis. Samples for serum 25(OH)D testing obtained at 6 and 12 monthswere analyzed at the end of the trial for blinding purposes. Long-term storage does not affect serum 25(OH)D, as reported by several studies (Agborsangaya et al., 2010; Colak, Toprak, Dogan, & Ustuner, 2013; Lewis & Elder, 2008; Lissner et al., 1981).

Vitamin D concentration was analyzed using a vitamin D (25-OH) assay via an electrochemiluminescence immunoassay (ECLIA) on the Cobas E-411 analyzer. Serum PTH was also analyzed on the Cobas E-411 analyzerusing ECLIA PTH.

The Dimension Vista® clinical chemistry system was used to analyze serum calcium and to obtain quantitative measurements of total cholesterol, HDL cholesterol, triglycerides and blood glucose. LDL cholesterol was not measured quantitatively but was calculated using the Friedewald equation with a simple mathematical equation: LDL = total cholesterol – HDL – TG / 2.2 (Friedewald, Levy, & Fredrickson, 1972). The results for LDL were prepared by the CDL. The blood samples were analysed for TC, HDL, LDL and TG however only HDL, LDL and TG were reported as part of the study. TC were not reported nor included in the data analysis as TC were not part of the formal metabolic syndrome definition.

Blood glucose was measured using the same Dimension Vista® clinical chemistry system based on the standard enzymatic reference hexokinase-glucose-6-phosphate dehydrogenase method. Blood insulin was analyzed using an IMMULITE 2000 system analyzer. Insulin resistance is known to be a predictor of type 2 diabetes mellitus, and HOMA-IR is commonly used to measure insulin resistance (Matthews et al., 1985).

3.12.3 Blood pressure (BP)

BP was measured twice using a digital sphygmomanometer (OMRON HEM-907 model). The average of the two measurements was recorded.

3.12.4 Anthropometric measurements

Participants' weight and height were measured using calibrated digital weighing scales and a stadiometer, respectively. Body weight was measured with a digital weighing scale

(Seca 808, Germany) to the nearest 0.1 kg with the participant wearing light clothing and no footwear. Height was measured using a stadiometer (Seca, Germany) to the nearest 0.1 cm. BMI was calculated with the formula of weight (kg)/height^2(metres). An elastic measuring tape was used to measure waist circumference (WC). The waist was measured at a point midway between the iliac crest and the costal margin (lower rib) with the participant in a standing position and after expiration, as recommended by WHO (WHO, 1995). All of the measurements were obtained to the nearest 0.1 cm. Regular meetings with the research assistants were conducted to ensure that standard protocols were followed.

3.12.5 SF-36v2 health-related quality of life (HRQOL) questionnaire

A validated self-administered questionnaire, the SF-36® version 2 (SF-36v2) health survey, was used to assess the participants' HRQOL in terms of physical and emotional well-being. The SF-36v2 was administered at baseline and at the12-month follow-up (pre- and post-treatment). Permission to use this questionnaire was purchased from QualityMetric SF™.

This health survey, which contains 36 questions measuring functional health and wellbeing from the participant's point of view, is a practical, reliable and valid measure of physical and mental health. The SF-36v2 is divided into eight domains or components, which are physical functioning, physical role functioning, bodily pain, general health, vitality, social role functioning, emotional role functioning and mental health, as well as a psychometrically based physical component summary (PCS) and a mental component summary (MCS). The QualityMetric SF™ smart measurement system was used to automatically calculate the scores. The scores at baseline and 12 months were then exported to SPSS software for further analysis. The SF36 was available in Malay and

English versions, and the participants were allowed to complete either version at their convenience.

The Malay version was validated for use in a Malaysian population aged ≥ 18 years by Sararaks et al. (2005). Most domains had high Cronbach's α (> 0.70), except for social functioning. Lower physical functioning, physical role functioning and bodily pain scores were observed with increasing age, which supports the construct validity of the SF-36 Malay version. In conclusion, the Malay version of SF-36 could be used in Malaysia due to its generally acceptable internal consistency and validity.

3.13 Lifestyles practices assessment

Lifestyles practices, such as dietary intake and physical activity, were measured because these factors are known confounders of cardiometabolic risk. Sunlight exposure, sunlight avoidance and vitamin D from dietary intake are known confounders of serum 25(OH)D concentration. These factors were measured two times, at baseline and 12 months, to ensure that an adjustment could be performed if any of the factors were found to change significantly throughout the trial.

There were three self-administered questionnaires assessing lifestyle practices. All three questionnaires were administered at baseline and the 12-month follow-up (pre- and post-treatment). The sunlight exposure history and IPAQ questionnaires were completed and returned to the researcher on the same day they were provided. The 7-day dietary recall was completed and returned to the researcher within two weeks.

3.13.1 Sunlight exposures history

The sunlight exposure history questionnaire was written in the Malay language. This questionnaire was pre-tested in individuals with racial and educational backgrounds similar

to a previous study conducted at the same setting (Moy, 2011). This questionnaire was administered to assess the participants' exposure to sunlight. It comprised two parts, sunlight exposure and sunlight avoidance. Sunlight exposure was assessed as the duration of outdoor activity or sun exposure in minutes per day per week. The sunlight exposure score was derived by multiplying the duration of sun exposure in minutes per day by the number of days spent outdoors (exposed to the sun) per week. To calculate the sunlight avoidance score, the participants were asked about their clothing style, such as wearing long sleeves or a veil (hijab), and the usage of umbrellas or sunscreens that block UVB light from penetrating the skin, thus preventing the formation of vitamin D in the skin. The sun avoidance score was calculated as the sum of the usage of sunscreens, veils, caps/hats, long-sleeved shirts / blouses, gloves, long pants / skirts and umbrellas (max = 7, min = 0). As Malaysia is a tropical country blessed with sunlight throughout the year, no questions were asked on the latitude or season of the year. A reliability and validation study conducted at the same setting produced mean (standard deviation) scores for the pre- and post-tests of 3.07 (1.08) and 2.94 (1.23), respectively ($p>0.05$). The correlation coefficient was 0.57 ($p<0.001$), Cronbach's α =0.72, and the intra-class correlation was 0.72 (95%CI: 0.52, 0.84) (Moy, 2014)

3.13.2 International Physical Activity Questionnaire (IPAQ)

The international physical activity questionnaire (IPAQ) was used to assess the level of physical activity of the participants. The 7-day long-form version of IPAQ was made available in both Malay (IPAQ-M) and English versions for the convenience of the participants, who were asked to recall the type and duration of their physical activity. IPAQ-M was validated for use in a Malaysian population (A. H. Chu & Moy, 2013). by researchers who recruited participants from the same study area as our research and found

that IPAQ-M had good reliability and validity for assessing physical activity in healthy Malay adults. The intra-class classification scores on items categorized by intensities and domains revealed moderate to good correlations (ICC: 0.54 to 0.92; $p < 0.001$). They also obtained a high kappa index ($k = 0.73$) for total activity and for all of the domains between the IPAQ-M and Physical activity Log (PA-Log).

IPAQ comprises four domains: leisure-time physical activities, domestic and gardening activities, work-related physical activities, and transport-related physical activities. The data collected were presented as continuous measures and reported as medians and interquartile ranges (IQR) in metabolic equivalent (MET)-minutes (METs). The following MET-minute values were used: walking = 3.3 METs; cycling for transportation = 6.0 METs; moderate physical activity = 4.0 METs, and vigorous physical activity = 8.0 METs. The total scores in MET-minutes per week was derived by summing the MET level x minutes of activity per day x days per week. Domain subscores were calculated using the MET-minute values, which were work, transportation, domestic and garden and leisure time. Total scores were calculated for walking, moderate-intensity activity, vigorous-intensity activity and total physical activity according to the IPAQ guidelines for scoring protocol (IPAQ Research Committee, 2005). The total physical activity scores obtained were classified into three categories for each participant as a low, moderate or high physical activity level according to the scoring protocol (Table 3.2).

Table 3.2: Categorical Score for Physical Activity (IPAQ)

	Categorical score	Criteria
1	Low	No activity reported **OR** some activity is reported but not enough to meet category 2 or 3
2	Moderate	Either of the following 3 criteria i. 3 or more days of vigorous-intensity activity of at least 20 minutes per day **OR** ii. 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day **OR** iii. 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week.
3	High	Any one of the following 2 criteria i. Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week **OR** ii. 7 or more days of any combination of walking, moderate- or vigorous- intensity activities accumulating at least 3000 MET-minutes/week

3.13.3 7-days dietary record

A 7-day dietary record was used to assess the energy and nutrient intakes of the participants. This method was selected as the method of choice for this trial because it was able to capture the participants' habitual eating patterns over two weekend days and five weekday days and to ensure that vitamin D intake was captured.

The participants were asked to complete a 24-hourdietary record for seven days in an open-entry form booklet (Appendix H). They were provided with verbal instructions for the completion of the record by a trained dietician. A list of written instructions with pictures of food models and serving sizes was also provided to them for reference during the briefing sessions. The participants were asked to provide a detailed description of the foods eaten (with brand names if applicable) and to estimate the amounts using standard serving

sizes (e.g., pieces or slices) or household measures (e.g., tablespoon or cup). The time of eating and preparation method were asked for, if applicable. After completing the dietary record, the participants were asked to return it to the researcher within two weeks of baseline and at the 12-month follow-up.

A standardized protocol was used to convert the estimated amounts into weights, and the data were entered into Nutritionist Pro™ Software version 2.0 from First DataBank, Inc., San Bruno, California, United States. The nutrient contents of the food items were obtained from the Nutrient Composition of Malaysian Foods database (Tee ES, 1997). Because the Nutrient Composition of Malaysian Foods did not include vitamin D nutrient, the United States Department of Agriculture (USDA) Standard Reference Database was used to estimate the vitamin D contents of raw foods. The vitamin D contents of commercial products were obtained from product labels if available because the labelling of vitamin D is optional in Malaysia. The total vitamin D intake (IU/day) was determined by multiplying the vitamin D content of the food, portion size and frequency of consumption. The consumption levels of total calories, vitamin D, and calcium as well as the proportions of the total calories comprising macronutrients, such as carbohydrates, protein and fat, were calculated.

3.14 Provision of results to participants

At baseline, all of the participants received the results of their anthropometric measurements, blood pressure, vitamin D level, lipid profiles and blood glucose. Upon completion, all of the participants were informed of their results at baseline and at the end of the trial. They were also informed whether they had been given active supplements or placebo. At the end of the trial, all of the participants in the placebo group were provided vitamin D supplements of 50,000 IU per week for eight weeks and 50,000 IU per month for

two months, and the participants who had taken the active dose were provided an additional 50,000 IU per month of the vitamin D supplements for three months.

3.15 Trial registration and ethical approval

Approval was obtained from the Ethics Committee of the University of Malaya Medical Centre (reference number 907.22; Appendix I), which governs all research projects involving human subjects conducted at the Medical Faculty of University of Malaya. The trial was registered with The Australian New Zealand Clinical Trial Registry (ACTRN12612000452897).

3.16 Data analysis

The participants' demographic data, such as name and address details, were maintained in Microsoft Access. A check box was used to record the progress of each participant throughout the trial period to allow for easy access and follow-up purposes. Raw data obtained from the questionnaires and measurements were entered into the statistical analysis software SPSS version 16.0 (SPSS Inc., 2009, Chicago, Illinois). The entered data were cleaned and checked for coding errors using random and consistent methods. Then, outliers and extreme values were checked.

Descriptive statistics were used for baseline characteristics. Categorical data were described using counts and percentages. All of the numerical data were checked for normality using the normal distribution test or test of normality. Normally distributed variables were presented as the means ± standard deviation, whereas abnormally distributed variables were presented as medians and interquartile ranges (IQR). Statistical comparisons of the baseline data between the intervention and placebo groups were performed with an independent *t*-test, theMann-Whitney U test or a Chi-square test, as appropriate.

The data were analyzed on an intention-to-treat (ITT) principle. Each outcome measure was analyzed at three time points (baseline and six and 12 months), except for HRQOL, sunlight exposure, IPAQ and dietary intake, which were analyzed at two time points (baseline and 12 months). To analyze the time course of outcome measures regarding exposure to the intervention or placebo, we used a generalized linear mixed model for repeated measures. Because a generalized linear mixed model was used for the repeated measures analysis, no imputation for any missing data was performed. Available data from participants with missing data were included in the mixed-model analysis and were assumed to be missing at random. The generalized linear mixed model method was applied to take into account the correlation between repeated measurements within the same participant. A random effects mixed model with unstructured covariate matrix was applied to test for interactions between variables. The multivariate model contained the outcome measures and treatment group as dependent variables, and the number of repeated measures (time of follow-up) was an independent variable. The independent variables were first analyzed separately and then analyzed for interactions between treatment and time of follow-up.

A sensitivity analysis using complete cases was also performed using the same generalized linear mixed model. In this analysis, only data from participants with complete data throughout the 12-month follow-up were included. Therefore, outcome data were obtained from only 171 out of 192 participants. Linear mixed models were performed as described above.

The significance level was pre-set at 0.05, and 95% confidence intervals were reported where appropriate. All of the statistical analyses were performed using SPSS software version 16.0 (SPSS Inc, 2009, Chicago, Illinois).

CHAPTER 4 : RESULTS

The results will be presented following the sequence of the specific objectives, as in section1.10.2. This chapter will be divided into three sub-sections.

Sub-section 4.1 describes the following baseline information:

a) Socio-demographic characteristics of the participants, including history of any chronic diseases
b) Lifestyle practices (dietary intake, physical activity and sunlight exposure)
c) Anthropometric, clinical and biochemical measurements
d) Health-related quality of life

Sub-section 4.2 describes the evaluation process throughout the intervention:

a) Response rate during recruitment
b) Characteristics of complete cases and participants who dropped out
c) Attendance at measurement sessions and intervention programmes

Sub-section 4.3 describes the post-intervention outcome evaluation:

a) Anthropometric, clinical and biochemical measurements
b) Health-related quality of life
c) Lifestyle practices (dietary intake, physical activity and sunlight exposure)

4.1 Baseline information

4.1.1 Socio-demographic characteristics of participants

The intervention and placebo groups consisted of 93 and 99 women, respectively, with Malays being the majority (n = 83 or 89.2% for the intervention group and n = 89 or 89.9% for the placebo group).

A majority of the participants had at least a secondary education. The mean age of the intervention group was similar (42.58 ± 5.35 years) to that of the placebo group (42.88 ± 4.99 years) (p = 0.69). There were 17 (8.9%) premenopausal participants aged ≥ 50 years, and more than 50% of them were aged between 40 and 49 years. A majority of the participants had occupations that required them to work indoors, such as administrative workers, lecturers and nurses (n = 75 or 80.6% for the intervention group and n = 84 or 84.8% for the placebo group). The participants who worked as general workers, such as gardeners or cleaners, usually spent more than 50% of their working hours in the sun. The intervention group consisted of 18 (19.4%) participants employed as general workers, whereas 15 (15.2%) general workers were in the placebo group. There were no differences between the two groups in terms of race, occupation or level of education. Table 4.1 shows the above information in greater detail.

Table 4.1: Socio-demographic characteristics of the intervention and placebo groups

	Intervention (n = 93)	**Placebo (n = 99)**
	Mean ± SD	**Mean ± SD**
Age (years)	42.58 ± 5.35	42.88 ± 4.99
	n (%)	**n (%)**
Age group (years)		
30 to 39	30 (32.3%)	33 (33.3%)
40 to 49	44 (59.1%)	57 (57.6%)
≥ 50	8 (8.6%)	9 (9.1%)
Ethnicity		
Malay	83 (89.3%)	89 (89.9%)
Chinese	3 (3.2%)	3 (3.0%)
Indian	7 (7.5%)	6 (6.1%)
Others	0 (0%)	1 (1.0%)
Level of education		
Primary	6 (6.5%)	8 (8.1%)
Secondary	43 (46.2%)	38 (38.4%)
Tertiary	44 (47.3%)	53 (53.5%)
Occupation		
Administrative staff	60 (64.5%)	59 (59.6%)
Lecturers	9 (9.7%)	22 (22.2%)
Nurses	6 (6.5%)	3 (3.0%)
General workers	18 (19.4%)	15 (15.2%)

4.1.2 History of chronic disease

The participants self-reported any history of chronic diseases, such as high cholesterol, hypertension or diabetes mellitus, diagnosed by doctors. A total of 15.6% of the participants had high cholesterol, 8.9% had hypertension, and only 1% had diabetes mellitus. There were comparable proportions of participants with a history of chronic diseases in both groups (Table 4.2)

Table 4.2: History of chronic disease in the intervention and placebo groups

History of chronic disease	Intervention (n = 93)	Placebo (n = 99)
	n (%)	**n (%)**
Diabetes mellitus	1 (1.1%)	1 (1.0%)
Hypertension	9 (9.7%)	8 (8.1%)
High-cholesterol	15 (16.1%)	15 (15.2%)
Metabolic syndrome	26 (28%)	22 (22.2%)

4.1.3 Anthropometric, clinical and biochemical measurements

Both groups were comparable in all the reported measures ($p > 0.05$) as shown in Table 4.3. Blood pressure was within the normal range for both groups. There were no differences in the serum PTH or calcium level between the groups. Similar observations were noted with regard to lipid profiles, blood glucose, blood insulin and HOMA-IR levels.

Table 4.3: Baseline anthropometric, clinical and biochemical measurements of the intervention and placebo groups

	Intervention (n = 93)	Placebo (n = 99)	Intervention (n=93)	Placebo (n=99)
			Abnormal rate (%)	
	Mean ± SD	**Mean ± SD**		
Height (cm)	1.56 ± 0.06	1.55 ± 0.06		
Weight (kg)	66.33 ± 12.22	65.66 ± 11.75		
BMI (kg/m^2)	27.23 ± 5.49	27.23 ± 5.09		
WC (cm)	32.75 ± 4.10	32.55 ± 3.63		
SBP (mmHg)	121.61 ± 16.19	118.90 ± 14.85	28 (30.1%)	23 (23.2%)
DBP (mmHg)	77.77 ± 10.64	76.80 ± 10.99	23 (24.7%)	23 (23.2%)
25(OH)D (nmol/l)	30.19 ± 8.71	29.99 ± 9.70		
Calcium (mmol/l)	2.18 ± 0.07	2.19 ± 0.08		
PTH (pmol/l)	4.60 ± 2.19	4.76 ± 2.05		
HDL	1.45 ± 0.49	1.44 ± 0.35	40 (43.0%)	34 (34.3%)
LDL	3.26 ± 0.76	3.34 ± 0.71	74 (79.6%)	86 (86.9%)
TG	1.15 ± 0.53	1.17 ± 0.62	16 (17.2)	18 (18.2%)
	Median; IQR	**Median; IQR**		
Glucose (mmol/l)	4.80; 0.65	4.80; 0.70	14 (15.1%)	9 (9.1%)
Insulin (mU/L)	9.30; 9.95	9.20; 8.80		
HOMA-IR	2.00; 2.28	2.00; 2.00		

The measurements were categorized as normal or abnormal according to the cut-off points defined in section 3.4, and the normal and abnormal rates are shown in Table 4.3. A majority of the participants had a high LDL level; less than 25% of both the intervention and placebo groups had a normal LDL level (LDL < 2.6 mmol/l). However, less than 50% of the participants in both groups had abnormal HDL or TG levels. Only 15.1% of the participants in the intervention group and 12.1% in the placebo group had blood glucose ≥ 5.6 mmol/l. Half of the participants in both groups had high insulin resistance. No significant differences ($p > 0.05$) were observed for any of these parameters.

Regarding BMI, only 23.7% of the participants in the intervention group and 21.2% in the placebo group were of normal weight (BMI 18.5 to 22.99 kg/m^2) (Figure 4.1). A BMI ≥

23 kg/m^2 was observed in 71 (76.3%) participants in the intervention group and 78 (78.8%) participants in the placebo group. Almost 50% of the participants in both groups were obese (BMI ≥ 27 kg/m^2).

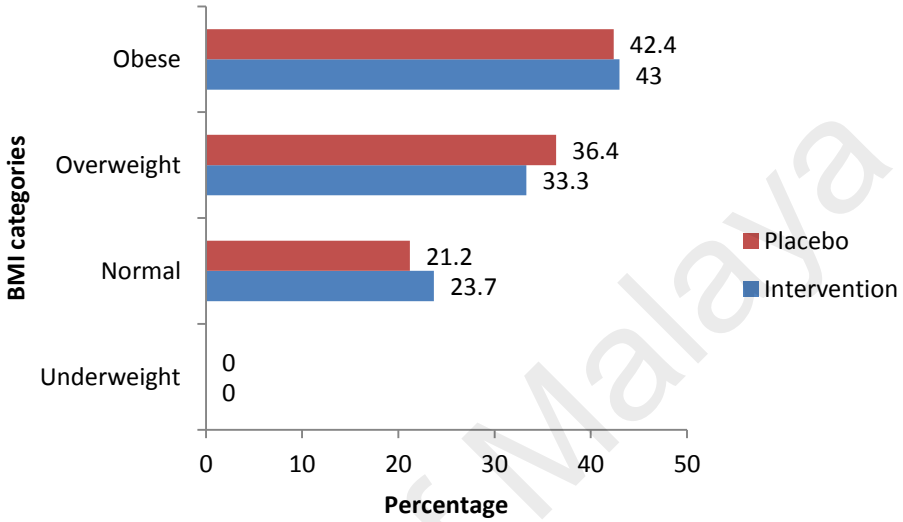

Figure 4.1: Comparison of BMI categories between groups

Figure 4.2 shows a comparison of metabolic syndrome characteristics between the groups. More than half of the participants were centrally obese, and 42% of the participants in the intervention group and 33% in the placebo group had low HDL or were receiving treatment for low HDL. A total of 12.9% in the intervention group and 15.2% in the placebo group had high TG or were receiving treatment for high TG. More than half of the participants were receiving treatment for hypertension or had high BP (≥130/85 mmHg), with 36.6% of them in the intervention group and 29.3% in the placebo group. The proportions of participants with metabolic syndrome were comparable in both groups ($p > 0.05$).

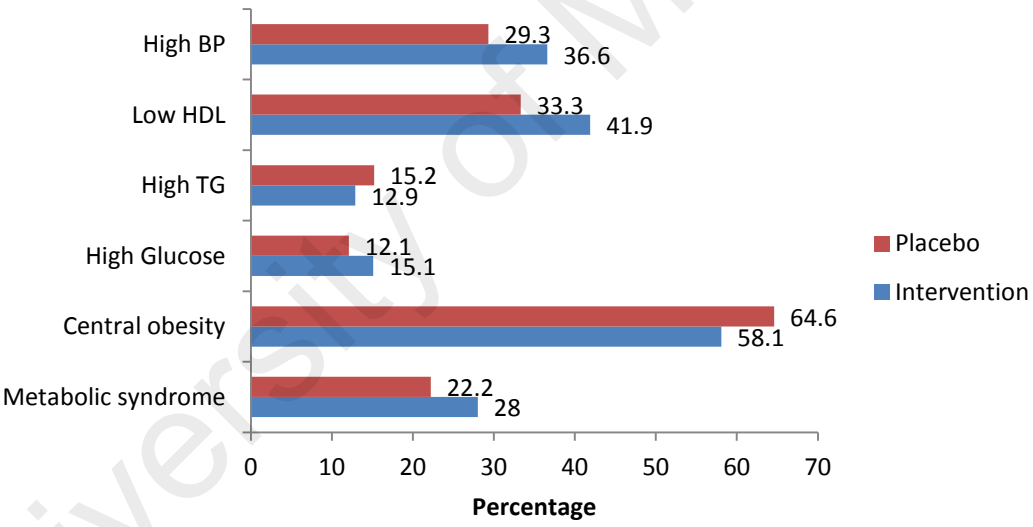

Figure **4.2**: Comparison of metabolic syndrome risk factors[1] between groups

[1]The metabolic syndrome risk factors considered were central obesity, whichwas defined as afemale waist circumference ≥ 80 cm, fasting blood TG ≥ 1.7 mmol/l or taking medication for high TG;a low HDL level (female below1.3 mmol/l) or taking medication for low HDL; elevated blood pressure (SBP ≥ 130 mmHg and/or DBP ≥ 85 mmHg) or taking antihypertensive medication; and fasting blood sugar≥ 5.6 mmol/l or taking medication for diabetes mellitus.

4.1.4 Health-related quality of life (HRQOL)

The mean scores for all of the HRQOL domains ranged from 60 to 70 (Table 4.4). The mean scores on all of the HRQOL domains were comparable between the groups ($p < 0.05$). The physical component summary (PCS) scoreand mental component summary (MCS) score were also comparable between the groups.

Table 4.4: Baseline health-related quality of life parameters of the intervention and placebo groups

	Intervention (n = 93)	**Placebo (n = 99)**
Physical functioning	74.68 ± 20.23	70.96 ± 24.24
Role physical	74.35 ± 22.98	72.27 ± 24.40
Bodily pain	73.13 ± 19.64	71.13 ± 18.31
General health	66.77 ± 16.87	66.12 ± 17.13
Vitality	65.27 ± 11.98	61.76 ± 13.88
Social functioning	78.77 ± 19.50	74.26 ± 21.85
Role emotional	78.92 ± 22.37	76.26 ± 23.99
Mental health	73.12 ± 12.11	71.06 ± 16.62
PCS	49.57 ± 6.78	48.80 ± 7.41
MCS	50.09 ± 6.69	48.65 ± 9.17

4.1.5 Lifestyles practices

4.1.5.1 Dietary intake

The dietary intake of the participants was assessed using a 24-hour dietary record for seven days. The dietary intakes of the participants are shown in Table 4.5. The difference in energy intake between the treatment groups was not significant ($p > 0.05$). The protein, carbohydrate and total fat intakes were quite similar in both groups. The contributions of macronutrients (% carbohydrate, % fat and % protein) to the total calorie intake were comparable between the intervention and placebo groups. Although the vitamin D intake levels were comparable between the intervention and placebo groups, only 22.7% (n = 22) of the intervention group and 29.3% (n = 29) of the placebo group met the recommended nutrient intake (RNI) for vitamin D. Calcium intake was comparable between the treatment groups ($p > 0.05$).

Table 4.5: Seven days dietary intake of the intervention and placebo groups at baseline

	Intervention (n = 93)	**Placebo (n = 99)**
	Mean ± SD	**Mean ± SD**
% Protein	15.3 ± 2.6	15.4 ± 2.5
% Carbohydrate	51.1 ± 6.1	51.5 ± 5.6
% Fat	33.3 ± 4.8	33.1 ± 4.8
	Median; IQR	**Median; IQR**
Energy (kcal/d)	1582.2; 374.7	1568.9; 476.3
Protein (g/d)	61.6; 20.5	59.7; 22.3
Carbohydrate (g/d)	201.8; 60.9	201.7; 80.1
Fat (g/d)	57.5; 21.3	58.4; 24.3
Vitamin D (IU/d)	155.3; 73.7	143.9; 101.6
Calcium (mg/d)	420.2; 200.9	423.5; 193.9
	n (%)	**n (%)**
Met RNI for vitamin D intake	22 (23.7%)	29 (29.3%)

4.1.5.2 Physical activity

Physical activity was assessed using the long form of the International Physical Activity Questionnaire (IPAQ). Overall, the physical activity levels of the participants in the placebo group and the intervention group were quite similar ($p > 0.05$). The most common physical activities performed by our participants were related to household chores or domestic work, including work in the garden. Less time was spent participating in recreational, sports or physical activities during leisure time. Participants from both treatment groups reported spending more than 300 minutes per day sitting, and the findings between the groups were comparable ($p > 0.05$). When physical activities were classified into categories, a high proportion of moderate physical activity was found in the participants from both treatment groups; there was no significant difference between the treatment groups in terms of physical activity categories ($p > 0.05$). Table 4.6 shows the above results in greater detail.

Table 4.6: Baseline IPAQ scores of the intervention and placebo groups at baseline

PA scores (MET-minutes / week)	**Intervention (n = 93)**	**Placebo (n = 99)**
	Median; IQR	**Median; IQR**
Work	495 (3442.5)	80 (2598)
Transportation	231 (495)	231 (495)
Domestic and garden	840 (1180)	805 (1535)
Leisure time	0 (396)	0 (3605)
Sitting (minutes/day)	342.9 (215.7)	328.6 (202.9)
Total walking scores	396 (1221)	396 (1287)
Total moderate scores	890 (1700)	840 (2070)
Total vigorous scores	240 (2400)	0 (1440)
Total PA scores	1857 (5844)	2108 (4390)
PA categories	**n (%)**	**n (%)**
Low	15 (16.1%)	21 (21.2%)
Moderate	50 (53.8%)	54 (54.5%)
High	28 (30.1%)	24 (24.2%)

PA: Physical activity

4.1.5.3 Sunlight exposure

Table 4.7 shows that the sunlight exposure scores and the sunlight protection scores were comparable between the groups ($p > 0.05$). Participants from both groups spent more than 40 minutes per week outdoors or exposed to sunlight.

Table 4.7: Sunlight exposure scores of the intervention and placebo groups at baseline

	Intervention (n = 93)	**Placebo (n = 99)**
	Mean ± SD	**Mean ± SD**
[a]Sunlight exposure scores	47.58 ± 73.51	43.38 ± 85.37
[b]Sunlight protection scores	3.08 ± 1.09	3.10 ± 0.97

[a]Minutes per week
[b]Sum of usage of sun block cream, veil, cap/hat, long sleeves shirt/blouse, gloves, long pants/skirt, umbrella (max = 7, min = 0)

A majority of the participants in the intervention and placebo groups (71.0% of the intervention group and 80.8% of the placebo group) wore a veil or hijab because the majority of them were Muslim, and a concealing style of dress was required by their religion. Similarly, most of the participants wore long shirts or blouses as well as long pants or a skirt. However, only 53.8% of the intervention group and 35.4% of the placebo group used sunblock (Figure 4.3).

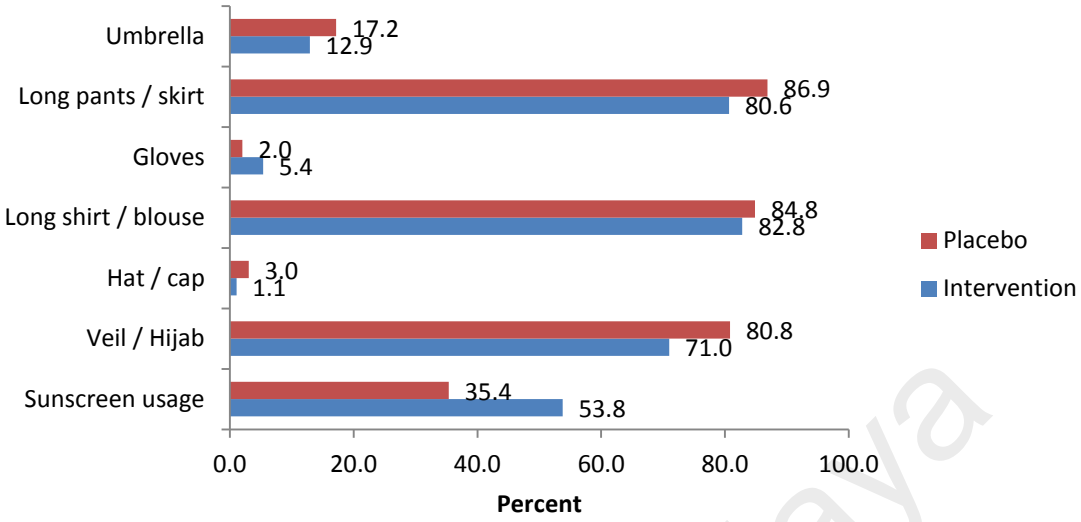

Figure 4.3: Comparison of the prevalence of sunlight protection use and clothing styles

4.2 Process evaluation

4.2.1 Response rate during screening

During the university's yearly health screening programme, from May 2012 to July 2012, 389 potentially suitable participants were identified and assessed for eligibility. Of these 389 candidates, 20 women were excluded due to menopause. Therefore, only 369 women underwent serum 25(OH)D analysis. Of these, 344 (93.2%) women were vitamin D deficient and were invited to participate in the study, whereas 22 (6%) had insufficient vitamin D status and only three (0.8%) had a sufficient vitamin D level.

4.2.2 Participant flow

Although all of the women who met the exclusion and inclusion criteria had received brief information regarding the study and had provided verbal consent before blood screening, 140 (40.7%) of them changed their mind and refused to participate when asked to give written consent before proceeding to phase two. Another five women (1.5%) were

pregnant at the time of phase two and were excluded before the randomization process. Two (0.6%) women were currently participating in other ongoing studies. Five (1.5%) women could not be contacted; they declined to answer phone calls or respond to letters. No reason was provided for not participating in the study.

Finally, out of 344 eligible women, only 192 (55.8%) consented to participate in the study. These participants were randomly assigned to either the intervention or placebo group. The randomization and intervention of the first cohort (n=110) commenced in October 2012 and ended in September 2013, whereas the second cohort (n=82) commenced in January 2013 and ended in December 2013. A total of 171 participants (89%) completed the follow-up at six months and 12 months. A flow diagram of the participants in the trial is shown in Figure 4.4.

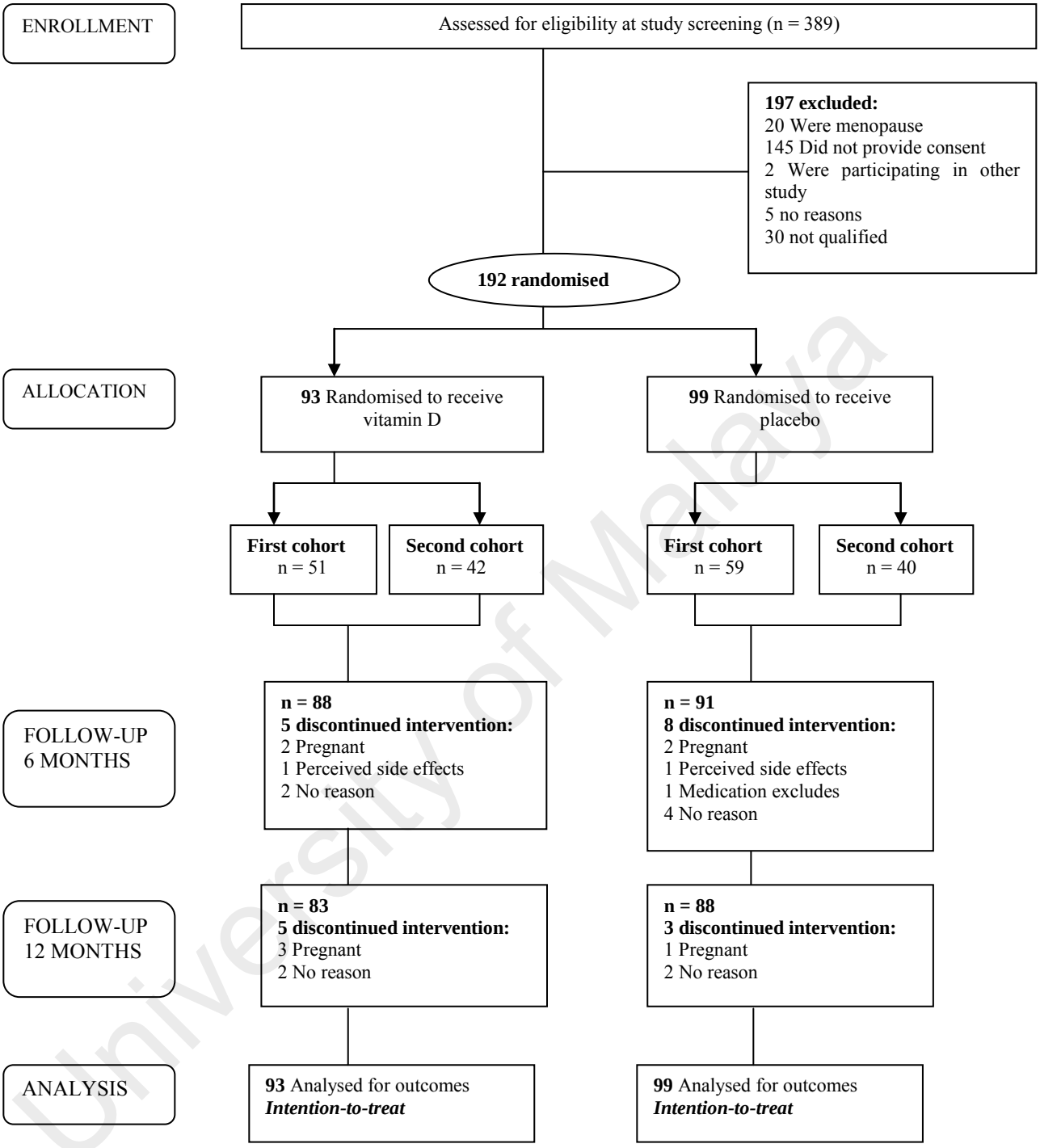

Figure 4.4: Flow diagram of the study, showing the numbers of participants who were randomly assigned, received the intended treatment and were analyzed for the primary outcomes

4.2.3 Resources distribution and intervention sessions

The health education activities and delivery of the intervention and placebo to the participants were handled by the researcher. Advice on the dietary aspect for the completion of the diet diary was managed by the co-investigator of this project.

During the 12-month follow-up, all of the participants were contacted through a mobile phone short message service (SMS), telephone or email. On first day of treatment, all of the participants were given a specially designed booklet (Appendix G), which contained the researcher's contact number, dates of the measurement sessions and intervention programme appointments as well as the signs and symptoms of vitamin D intoxication. Reminders were sent to every participant through email and SMS one week before the appointment date for the intervention programme or measurement sessions and were repeated one day before the appointment date. Participants who missed appointments or were unavailable on scheduled appointment dates were provided alternative dates suitable for them. Alternatively, the researcher made an effort to personally go to participants' work place to supply them with the active supplement or placebo within one week after the appointment date. SMS and phone calls were sent to remind the participants to consume the active supplement or placebo, and the participants were required to reply via SMS confirming that they had consumed the supplement or placebo.

The participants were required to complete all of the questionnaires during the appointments at baseline and 12 months. As a token of appreciation and to encourage the participants to complete their diet diary within two weeks, a recipe book was provided upon submission of the completed diary. The attendance at the measurement sessions and compliance with the treatment was 100%.

4.2.4 Response rate during the intervention programme

During the 12-month follow-up period, 21 (10.9%) women withdrew from the trial due to pregnancy, perceived side effects (amenorrhea), being prescribed vitamin D (≥ 1000 IU per day) by a medical doctor due to bone fracture, or for no specified reason. Only 10.8% and 11.1% of the participants dropped out from the intervention and placebo groups, respectively. Figure 4.5 shows the reasons for withdrawal by group.

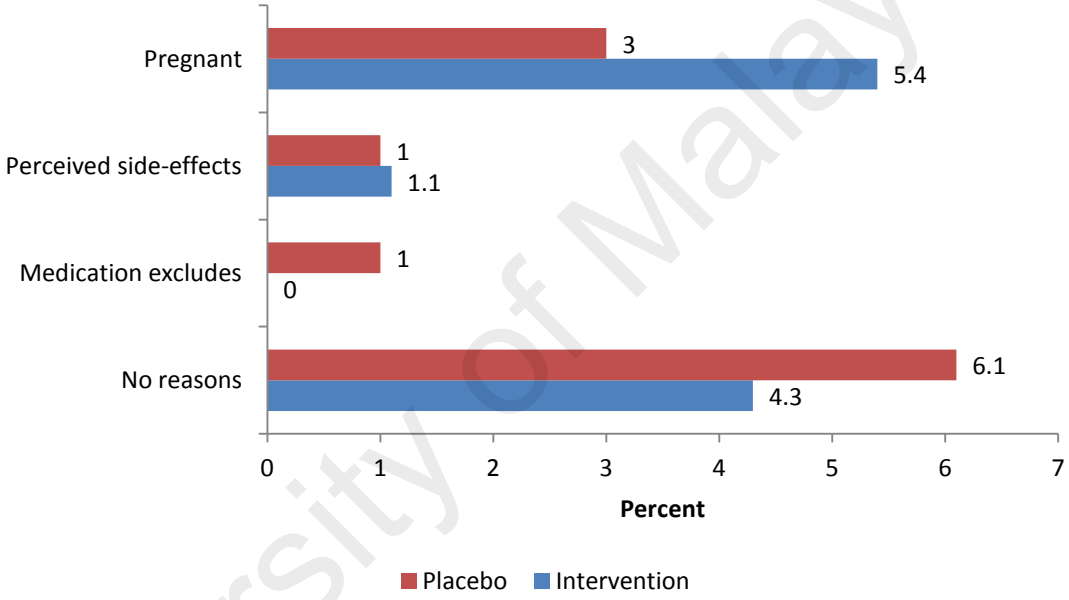

Figure 4.5: Reasons for loss to follow-up for both the intervention and placebo groups

Compared with the participants who completed the study, those who dropped out were significantly younger and had lower BMI and TG levels (Table 4.8). Otherwise, there were no differences in other baseline characteristics between the participants who completed the study and those who dropped out.

Table 4.8: Baseline characteristics of the participants who completed the study and those who dropped out

	Completed study (n = 171)	**Dropped out (n = 21)**	**p-value**
	n (%)	**n (%)**	
Race			
Malay	152 (88.9%)	20 (95.2%)	0.105[b]
Chinese	6 (3.5%)	0 (0%)	
Indian	13 (7.6%)	0 (0%)	
Others	0 (0%)	1 (4.8%)	
Occupation			
Lecturer	24 (14%)	7 (33.3%)	0.086[b]
Administrative staff	106 (62.0%)	13 (61.9%)	
Nurses	9 (5.3%)	0 (0%)	
General worker	32 (18.7%)	1 (4.8%)	
Level of education			
Primary	14 (8.2%)	0 (0%)	0.371[b]
Secondary	69 (40.4%)	11 (52.4%)	
Tertiary	88 (51.5%)	10 (47.6%)	
History of CVD[b]			
DM	2 (1.2%)	0 (0%)	0.793
HPT	14 (8.2%)	3 (14.3%)	0.279
HPL	28 (16.4%)	2 (9.5%)	0.327
Metabolic syndrome	31 (18.1%)	2 (9.5%)	0.259
	Mean ± SD	**Mean ± SD**	
Age	43.16 ± 5.06	39.24 ± 4.67	**0.001[a]**
BMI	27.5 ± 5.06	24.97 ± 6.48	**0.037[a]**
WC	32.8 ± 3.59	31.09 ± 5.42	**0.050[a]**
SBP	120.8 ± 15.2	115.8 ± 17.8	0.165
DBP	77.5 ± 10.5	75.8 ± 12.9	0.499
25(OH)D	30.29 ± 9.15	28.36 ± 9.71	0.363
HOMA-IR	3.19 ± 7.63	2.11 ± 1.73	0.519
TG	1.20 ± 0.58	0.89 ± 0.52	**0.018[a]**
HDL	1.47 ± 0.49	1.37 ± 0.28	0.390
LDL	3.26 ± 0.82	3.18 ± 1.04	0.698

[a] $p < 0.05$ versus the participants who completed the study
[b] Analysis determined with Fisher's Exact test

4.2.5 Reports of adverse reactions to the intervention

There were no reports of adverse reactions by the participants during or after the intervention programme. There were also no abnormal serum calcium results in the test for hypercalcaemia secondary to vitamin D intoxication at the six-month follow-up. Only two participants (one from the placebo group and one from the intervention group) reported having amenorrhea after starting the treatment. An endocrinologist from the University of Malaya Medical Centre was consulted on this issue, and he confirmed that amenorrhea is neither a side effect of vitamin D supplementation nor a symptom of vitamin D intoxication. Both participants were provided with this information; however, they still refused to continue with the programme.

4.3 Outcome evaluation

4.3.1 Biochemical measurements

Table 4.9 shows the outcomes of the biochemical measurements after 12 months of follow-up at six-month intervals. Both serum 25(OH)D and serum PTH were improved after 12 months of follow-up ($p < 0.001$). Other outcome measures were not significantly different between the groups, except TG, which was significantly increased at six months (mean difference: 0.19, 95% CI: 0.01 to 0.37 mmol/l). However, the effect was clinically insignificant. LDL was significantly increased within the treatment group for both the intervention (mean difference: 0.358, 95% CI: 0.148 to 0.568) and placebo groups (mean difference: 0.317, 95% CI: 0.147 to 0.487); however, the effect was clinically insignificant.

Table 4.9: Biochemical measurement of the intervention and placebo groups at baseline, sixth month and 12th month follow-ups

	Intervention (n = 93)	Placebo (n = 99)	Mean difference (95% CI) between groups[a]
	Mean (95% CI)	Mean (95% CI)	
25(OH)D (nmol/l)			
Baseline	30.19 (28.56 to 31.82)	29.98 (27.20 to 32.78)	0.19 (-2.43 to 2.82)
6 month	83.91 (79.84 to 87.97)	37.15 (34.39 to 39.90)	**46.92 (42.42 to 51.43)***
12 months	85.74 (81.25 to 90.22)	36.09 (33.63 to 38.55)	**49.54 (43.94 to 55.14)***
PTH (pmol/l)			
Baseline	4.60 (4.18 to 5.03)	4.76 (4.34 to 5.18)	-0.16 (-0.76 to 0.45)
6 month	4.58 (4.05 to 5.10)	5.86 (5.34 to 6.38)	**-1.28 (-2.02 to -0.55)***
12 months	4.19 (3.74 to 4.66)	5.22 (4.77 to 5.67)	**-1.02 (-1.67 to -0.38)***
Calcium (mmol/l)			
Baseline	2.18 (2.17 to 2.19)	2.19 (2.18 to 2.21)	-0.01 (-0.03 to 0.009)
6 month	2.23 (2.21 to 2.26)	2.24 (2.21 to 2.26)	-0.005 (-0.04 to 0.03)
12 months	2.24 (2.22 to 2.26)	2.22 (2.20 to 2.24)	0.02 ((-0.01 to 0.05)
Glucose (mmol/l)			
Baseline	5.07 (4.88 to 5.25)	4.93 (4.75 to 5.12)	0.13 (-0.13 to 0.39)
6 month	5.14 (4.97 to 5.32)	5.07 (4.89 to 5.24)	0.07 (-0.17 to 0.32)
12 months	5.04 (4.83 to 5.26)	5.11 (4.90 to 5.32)	-0.07 (-0.37 to 0.23)
Insulin (mU/L			
Baseline	13.81 (10.38 to 17.24)	11.07 (7.74 to 14.39)	2.74 (-2.04 to 7.51)
6 month	13.11 (11.18 to 15.04)	12.17 (10.27 to 14.06)	0.943 (-1.75 to 3.65)
12 months	13.93 (11.47 to 16.38)	12.74 (10.36 to 15.12)	1.19 (-2.23 to 4.61)
HOMA-IR			
Baseline	3.72 (2.25 to 5.19)	2.47 (1.04 to 3.91)	1.25 (-0.81 to 3.31)
6 month	3.12 (2.57 to 3.67)	2.84 (2.29 to 3.38)	0.28 (-0.49 to 1.05)
12 months	3.19 (2.61 to 3.78)	2.99 (2.42 to 3.56)	0.21 (-0.61 to 1.03)
LDL (mmol/l)			
Baseline	3.26 (3.11 to 3.41)	3.34 (3.19 to 3.48)	-0.08 (-0.29 to 0.13)
6 month	3.25 (3.09 to 3.39)	3.31 (3.17 to 3.46)	-0.07 (-0.28 to 0.15)
12 months	3.62 (3.44 to 3.79)	3.63 (3.46 to 3.80)	-0.01 (-0.26 to 0.23)
HDL (mmol/l)			
Baseline	1.45 (1.36 to 1.54)	1.44 (1.35 to 1.52)	0.01 (-0.11 to 0.13)
6 month	1.43 (1.36 to 1.49)	1.50 (1.44 to 1.57)	-0.08 (-0.18 to 0.02)
12 months	1.52 (1.44 to 1.59)	1.50 (1.43 to 1.58)	0.02 (-0.09 to 0.13)
TG (mmol/l)			
Baseline	1.15 (1.03 to 1.26)	1.17 (1.06 to 1.29)	-0.03 (-0.19 to 0.14)
6 month	1.38 (1.25 to 1.51)	1.19 (1.07 to 1.32)	**0.19 (0.01 to 0.37)***
12 months	1.36 (1.23 to 1.49)	1.22 (1.09 to 1.35)	0.14 (-0.33 to 0.33)

* Significant at $p < 0.05$ between treatment groups

[a] Determined using linear mixed effects model

Table 4.10 showed the changes in outcome variables within treatment group from baseline to 6-months and 12-months and mean differences in outcomes between treatment groups at 12-months. Serum 25(OH)D in both groups improved throughout the intervention and maintained significant improvement at 12-months compared to baseline ($p<0.001$). These changes were found to be significantly differed between treatment groups ($p<0.001$). Serum PTH significantly increased in placebo group from baseline to 6-months while in vitamin D group, there was slight but not significant improvement from baseline to 6 months and baseline to 12 months. However, there were differences between groups for serum PTH ($p = 0.047$). Both groups showed significant improvement in serum Ca however there were no differences between groups for serum Ca ($p = 0.296$). There were no differences in other outcomes between groups.

Table 4.10: Changes in outcome variables by treatment group from baseline to 6- and 12-months and differences in outcomes among the treatment groups at 12-months

Outcome	Month	Mean change from baseline (95% CI)[a]		Time		Group x time
		Vitamin D (n = 93)	**Placebo (n = 99)**	***P* value**	**95% CI[b]**	***P* value**
Se 25(OH)D	6	**-53.87 (-60.29 to -47.44)**	**-7.31 (-11.32 to -3.31)**			
	12	**-55.58 (-60.91 to -50.24)**	**-6.24 (-9.55 to -2.92)**	**<0.001**	**-49.34 (-55.50 to -43.15)**	**<0.001**
Se PTH	6	0.02 (-0.78 to 0.83)	**-1.07 (-1.9 to -0.28)**			
	12	0.40 (-0.26 to 1.07)	-0.47 (-1.12 to 0.19)	**0.047**	0.87 (-0.07 to 1.80)	**0.047**
Se Ca	6	**-0.05 (-0.09 to -0.01)**	**-0.04 (-0.08 to -0.01)**			
	12	**-0.06 (-0.09 to -0.03)**	**-0.03 (-0.05 to -0.0008)**	**<0.001**	-0.03 (-0.07 to 0.009)	0.296
HOMA-IR	6	0.61 (-1.7 to 2.9)	-0.36 (-1.19 to 0.46)			
	12	0.53 (-1.39 to 2.44)	-0.51 (-1.19 to 0.17)	0.964	1.04 (-0.94 to 3.02)	0.507
LDL	6	0.01 (-0.28 to 0.29)	0.01 (-0.24 to 0.26)			
	12	**-0.36 (-0.59 to -0.12)**	-0.29 (-0.49 to 0.09)	**<0.001**	-0.07 (-0.38 to 0.25)	0.891
HDL	6	0.02 (-0.12 to 0.17)	-0.06 (-0.18 to 0.05)			
	12	-0.07 (-0.19 to 0.05)	-0.06 (-0.16 to 0.03)	0.208	-0.007 (-0.16 to 0.15)	0.409
TG	6	**-0.24 (-0.47 to -0.006)**	-0.03 (-0.22 to 0.17)			
	12	**-0.22 (-0.41 to -0.02)**	-0.05 (-0.21 to 0.11)	0.052	-0.16 (-0.41 to 0.68)	0.204
SBP	6	-4.7 (-10.4 to 1.1)	-5.0 (-10.1 to 0.1)			
	12	-4.1 (-8.9 to 0.6)	**-4.9 (-9.2 to -0.7)**	**0.003**	0.82 (-5.5 to 7.2)	0.968
DBP	6	-1.2 (-5.9 to 2.0)	-2.4 (-6.1 to 1.3)			
	12	0.25 (-3.1 to 3.6)	0.04 (-3.0 to 3.1)	0.078	0.22 (-4.3 to 4.7)	0.983

[a]Time differences were calculated as (baseline - 6-month) and (baseline - 12-month)
[b]Mean differences between group at 12 months

4.3.1.1 Serum 25(OH)D

The mean serum 25(OH)D concentration in the intervention group increased drastically in the first six months (mean difference: -53.87, 95% CI: -60.29 to -47.44 nmol/l (Table 4.10). There was also a significant increase in the mean serum 25(OH)D in the placebo group after six months (mean difference: -7.31, 95% CI:-11.32 to -3.31nmol/l); however, the level was still categorized as deficient (<50 nmol/l). A total of 94% of the participants in the intervention group achieved a 25(OH)D level greater than 50 nmol/l (20 ng/ml), and 65% achieved a level of 75 nmol/l (30 ng/ml) or higher (Figure 4.6). In contrast, 15% of the placebo group had a 25(OH)D level above 50 nmol/l, and only 1% had a value of 75 nmol/l or higher.

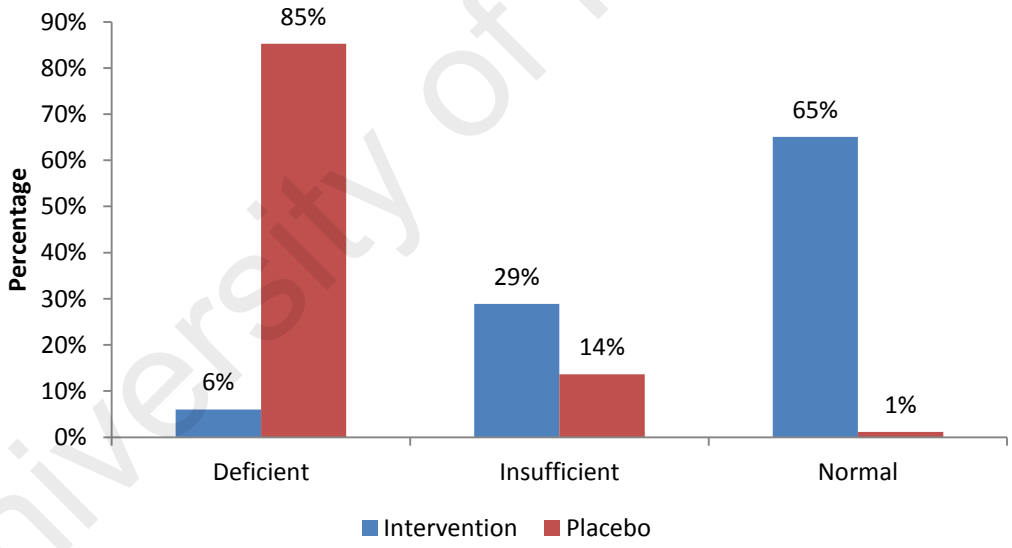

Figure 4.6: Comparison of the prevalence of vitamin D status between the treatment groups after 12 months of intervention

4.3.1.2 Serum PTH

The mean PTH concentration decreased in the intervention group from baseline to 12 month, but the change was not significant (mean difference: 0.40, 95% CI: -0.26 to 1.07 pmol/l) (Table 4.10 and Figure 4.7). In the placebo group, the mean PTH concentration increased from baseline to 12 months (mean difference: 0.47, 95% CI: -1.12 to 0.19 pmol/l). The change in PTH from baseline to 12 months was significantly different between the treatment groups (p = 0.047) (Table 4.10).

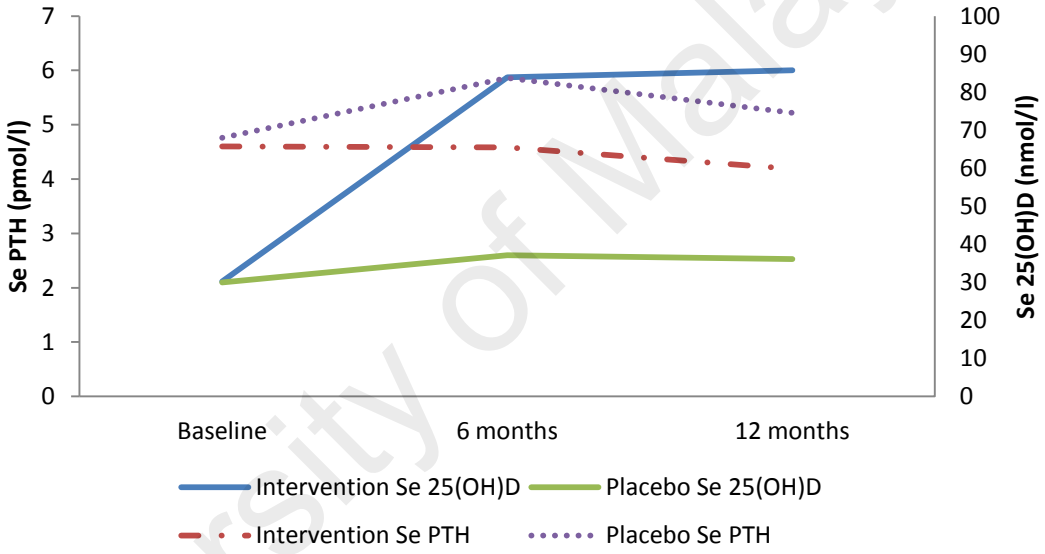

Figure 4.7: Mean serum 25(OH)D and PTH levels of the intervention and placebo groups over a 12 month period at 6 month intervals

4.3.1.3 Serum calcium

As the serum 25(OH)D level increased, the serum calcium level also improved in the first six months in the intervention group (mean difference: -0.05, 95% CI: -0.09 to -0.01 mmol/l), and it remained at a plateau between six and 12 months of follow-up (Table 4.10 and Figure 4.8). A similar increase was observed in the placebo group within the first six months (mean difference: -0.04, 95% CI: -0.08 to -0.01 mmol/l). However, after six months, the serum calcium level in the placebo group started to decrease. Nevertheless, there were no differences in mean serum calcium concentrations between the treatment groups over time (p = 0.296) (Table 4.10).

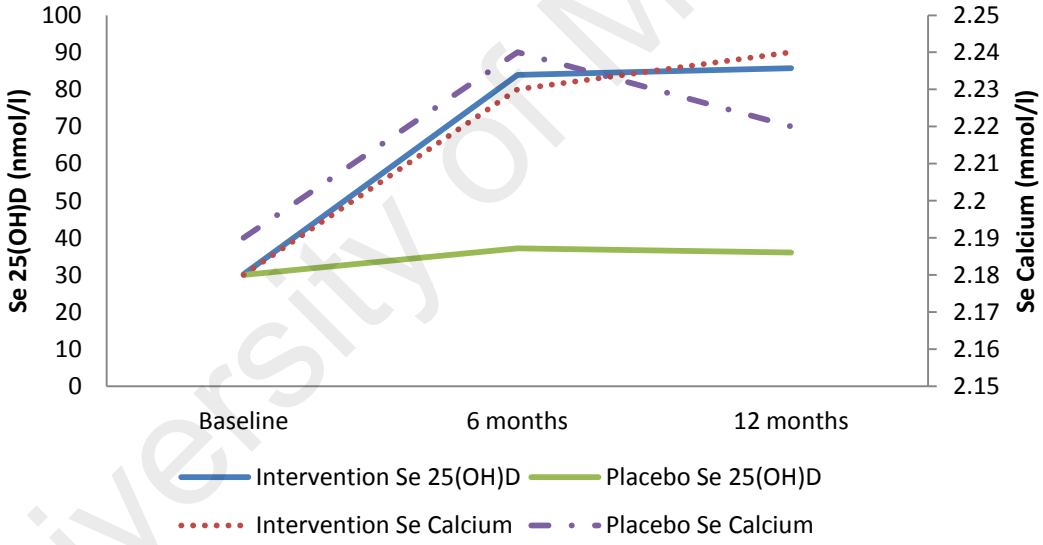

Figure 4.8: Mean serum 25(OH)D and calcium levels of the intervention and placebo groups over a 12 month period

4.3.1.4 Insulin resistance (HOMA-IR)

An increase in serum 25(OH)D in the intervention group induced a reduction in HOMA-IR from baseline to 12 months, whereas the reverse was observed in the placebo group (mean difference: 0.53, 95% CI: -1.39 to 2.44 versus mean difference: -0.51, 95% CI: -1.19 to 0.17) (Table 4.10 and Figure 4.9). However, these changes were not significant between or within the treatment groups over time (Table 4.10).

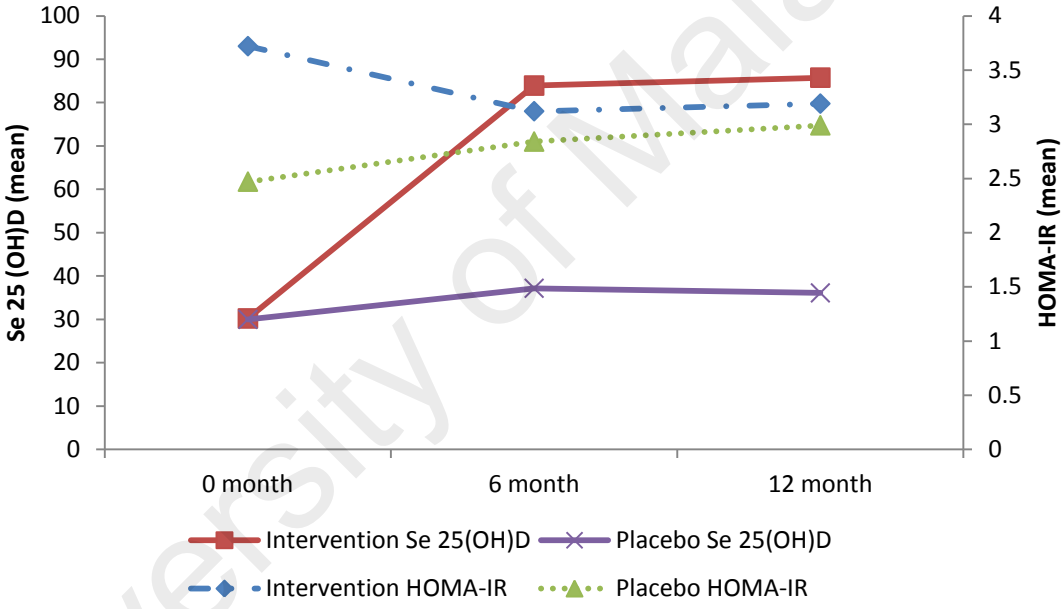

Figure 4.9: Mean in insulin resistance (HOMA-IR) and Se (25(OH)D of the intervention and placebo groups over 12 month period at 6 month intervals

4.3.1.5 Low-density lipoprotein (LDL)

There were no significant differences in LDL levels between the treatment groups over time (p = 0.891) (Table 4.10). A significant increase in the LDL level from baseline to 12 months of follow-up was observed in both the intervention group and non-significant increase in the placebo group (mean difference: -0.36, 95% CI: -0.59 to -0.12 mmol/l versus -0.29, 95% CI: -0.49 to 0.09 mmol/l, respectively) (Table 4.10 and Figure 4.10). However, the increase was clinically insignificant.

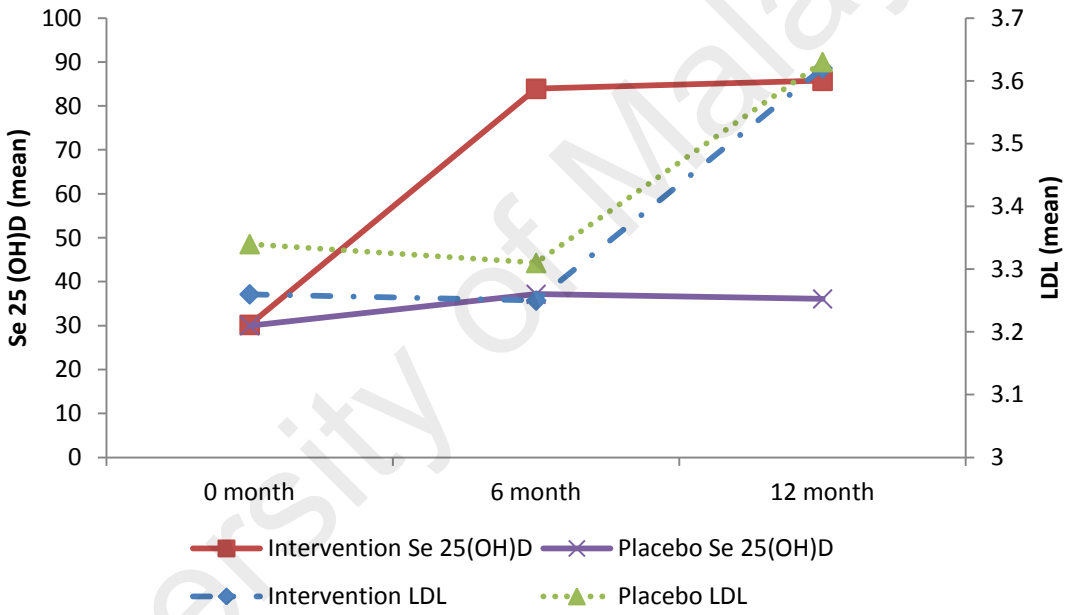

Figure 4.10: Means in the LDL levels (mmol/l) and Se 25(OH)D of the intervention and placebo groups over a 12 month period at 6 month intervals

4.3.1.6 High-density lipoprotein (HDL)

As the serum 25(OH)D increased in the intervention group in the first 6 months, the HDL level decreased marginally (mean difference: 0.02, 95% CI: -0.12 to 0.17 mmol/l) (Table 4.10 and Figure 4.11). However, after six months, the HDL level in the intervention group started to increase significantly (mean difference: 0.084, 95% CI: 0.006 to 0.162 mmol/l) (Figure 4.11). In the placebo group, some increase was observed from baseline to six months (mean difference: -0.06, 95% CI: -0.18 to 0.05 mmol/l); however, the HDL level plateaued from six months to 12 months of follow-up. Nevertheless, the changes showed no significant difference between the treatment groups over time (p = 0.409) (Table 4.10).

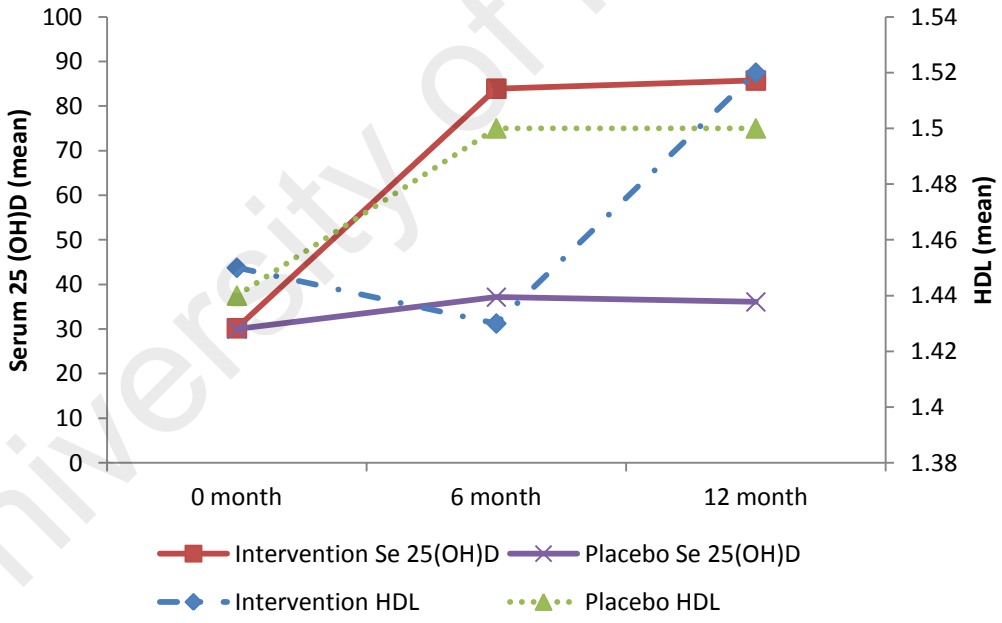

Figure 4.11: Mean in the HDL levels (mmol/l) and Se 25(OH)D of the intervention and placebo groups over 12 month period at 6 month intervals

4.3.1.7 Triglycerides (TG)

There were no significant differences in triglycerides between the treatment groups over time (p = 0.204) (Table 4.10). Figure 4.12 shows there was a significant increase from baseline to 12 months in the intervention group (mean difference: -0.22, 95% CI: -0.41 to -0.02 mmol/l) as the serum 25(OH)D increased. Compared to the placebo group, the TG level in the intervention group exhibited a plateau trend from baseline to 12 months (mean difference: -0.05, 95% CI: -0.21 to 0.11 mmol/l). Nevertheless, the triglyceride levels of both treatment groups were maintained within the normal range before and after the intervention.

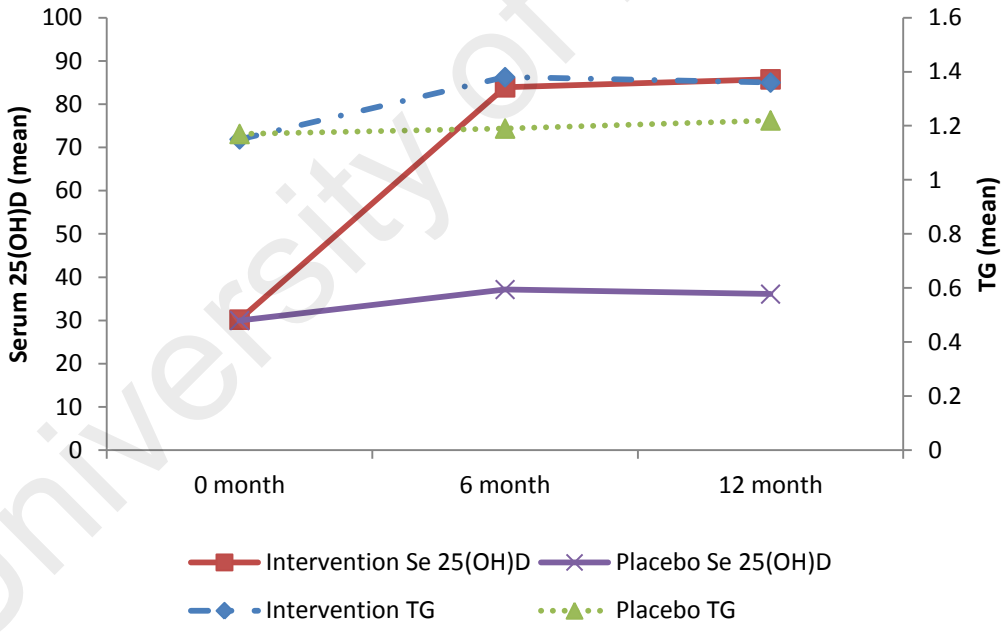

Figure 4.12: Mean in the triglycerides levels (mmol/l) and Se 25(OH)D of the intervention and placebo groups over a 12 month period at 6 month intervals

4.3.2 Blood pressure (BP)

Table 4.11 shows the BP measurements of both groups over 12 months of follow-up. Both the SBP and DBP values were normal at baseline.

Table 4.11: Blood pressure measurement of the intervention and placebo groups over 12 months period at 6 monthly intervals

	Intervention (n = 93)	Placebo (n = 99)	Mean difference (95% CI) between groups[a]
	Mean (95% CI)	Mean (95% CI)	
SBP (mmHg)			
Baseline	121.6 (118.4 to 124.8)	118.9 (115.8 to 121.9)	2.71 (-1.71 to 7.13)
6 month	126.3 (122.9 to 129.6)	123.9 (120.7 to 127.2)	2.38 (-2.27 to 7.04)
12 months	125.8 (122.6 to 128.9)	123.9 (120.8 to 126.9)	1.89 (-2.56 to 6.35)
DBP (mmHg)			
Baseline	77.77 (75.56 to 79.99)	76.79 (74.65 to 78.94)	0.976 (-2.107 to 4.059)
6 month	79.74 (77.38 to 82.10)	79.23 (76.97 to 81.55)	0.508 (-2.805 to 3.820)
12 months	77.52 (75.22 to 79.81)	76.76 (74.53 to 78.99)	0.757 (-2.441 to 3.954)

* Significant at $p < 0.05$ between treatment group
[a] Determined using linear mixed effect model

4.3.2.1 Systolic blood pressure (SBP)

In the intervention group, there was an insignificant increase in SBP from baseline to 12 months (mean difference: -4.1, 95% CI: -8.9 to 0.6 mmHg), whereas there was a significant increase in the placebo group (mean difference: -4.9, 95% CI: -9.2 to -0.7 mmHg). There were no significant differences in SBP between the groups over time ($p = 0.968$) (Table 4.10), and the readings were within the normal range.

4.3.2.2 Diastolic blood pressure (DBP)

The DBP measurements of the intervention and placebo groups showed a plateau trend from baseline to 12 months of follow-up (mean difference: 0.25, 95% CI: -3.1 to 3.6 mmHg and 0.04, 95% CI: -3.0 to 3.1 mmHg, respectively). There were no significant differences in DBP between the groups over time ($p = 0.983$) (Table 4.10), and the readings were within the normal range.

4.3.3 Indicators of obesity

Table 4.12 shows the BMI and WC measurements of the treatment groups at baseline and at the six- and 12-month follow-ups. The mean BMI levels at all three time points were found to be in the above-normal category, and no significant changes in BMI were observed within or between the treatment groups. Similarly, the mean WC was greater than 80 cm (indicating central obesity) at all three time points, with no significant changes found within or between the groups.

Table 4.12: Indicators of obesity measurements of the intervention and placebo groups over a 12 month period at 6 month intervals

	Intervention (n = 93)	Placebo (n = 99)	Mean difference (95% CI) between groups[a]
	Mean (95% CI)	Mean (95% CI)	
BMI (kg/m^2)			
Baseline	27.22 (26.14 to 28.31)	27.23 (26.18 to 28.28)	-0.005 (-1.511 to 1.500)
6 month	28.08 (26.96 to 29.19)	27.76 (26.67 to 28.86)	0.313 (-1.251 to 1.877)
12 month	27.63 (26.45 to 28.78)	27.84 (26.73 to 28.95)	-0.209 (-1.807 to 1.389)
WC (cm)			
Baseline	83.19 (81.05 to 85.34)	82.67 (80.82 to 84.51)	0.53 (-2.28 to 3.33)
6 month	85.78 (83.62 to 87.96)	85.29 (83.36 to 87.22)	0.50 (-2.39 to 3.38)
12 month	84.89 (82.63 to 87.15)	84.43 (82.27 to 86.59)	0.46 (-2.64 to 3.56)

* Significant at $p < 0.05$ between treatment group
[a] Determined using linear mixed effect model

4.3.3.1 Body mass index (BMI)

There was a non significant but escalating trend in BMI within the placebo group over time (Figure 4.13). In the intervention group, there was an increase in BMI from baseline to six months (mean difference: 0.559, 95% CI: -0.412 to 1.530 kg/m^2); however, the BMI was decreased at 12 months (mean difference: -0.304, 95% CI: -1.329 to 0.722 kg/m^2). Overall, there were no significant differences in BMI within or between the groups.

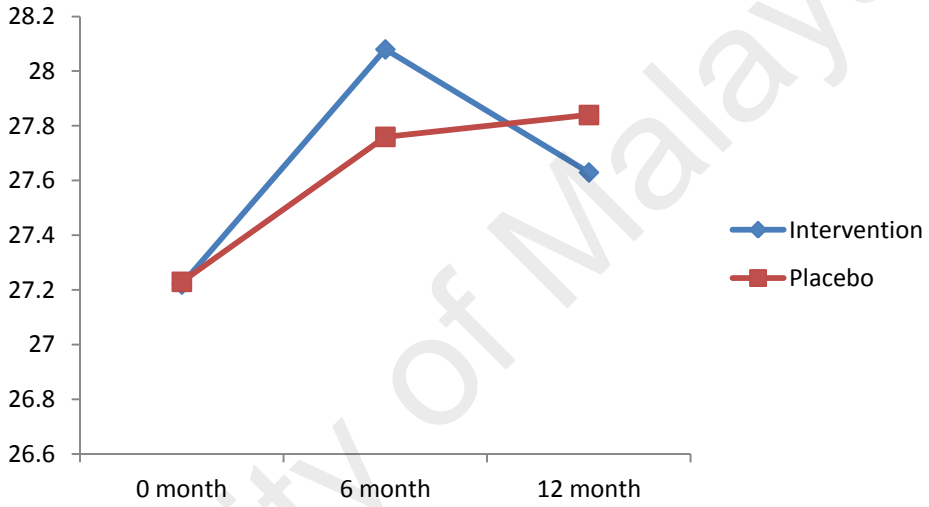

Figure 4.13: Mean in BMI (kg/m^2) of the intervention and placebo groups over a 12 month period at 6 month intervals

When the participants were categorized by BMI as normal weight (BMI 18.5 to 22.9 kg/m^2) or overweight (BMI ≥ 23 kg/m^2) and BMI was compared with vitamin status at 12 months, six out of seven overweight participants in the intervention group were still found to be vitamin D deficient despite 12 months of intervention with vitamin D supplements (Figure 4.14).

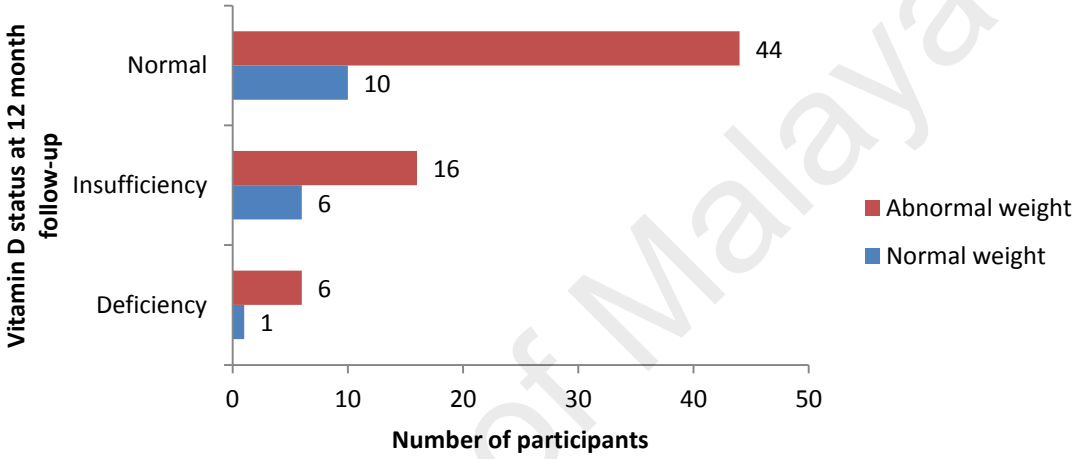

Figure 4.14: Comparison of prevalence of the BMI and vitamin D status in the intervention group at the 12 month follow-up

4.3.3.2 Waist circumference (WC)

There were no differences in the trends of WC readings at baseline, sixth and 12th month in either treatment group (Table 4.12).

4.3.4 Metabolic syndrome

Figure 4.15 shows the data concerning metabolic syndrome and its risk factors in the intervention and placebo groups at baseline and the 12-month follow-up. There was a small reduction (from 28% to 25.8%) in the proportion of participants with metabolic syndrome in the intervention group, whereas the proportion increased from 22.2% to 23.2% in the placebo group. There was no difference between the groups (p = 0.762). A majority of the

participants were centrally obese (WC > 80 cm) at baseline, and the WC measurements were increased at follow-up in both treatment groups. The proportions of participants with high BP and / or receiving treatment for hypertension increased in both the intervention and placebo groups, from 36.6% and 29.3% at baseline to 38.7% and 31.3% at follow-up, respectively. The participants who were receiving treatment for high cholesterol and / or had high TG increased in both treatment groups; however, the proportion of participants with a low HDL level who were receiving treatment was reduced from 41.9% to 36.6% in the intervention group and from 33.3% to 29.3% in the placebo group. However, the differences between the treatment groups were not significant.

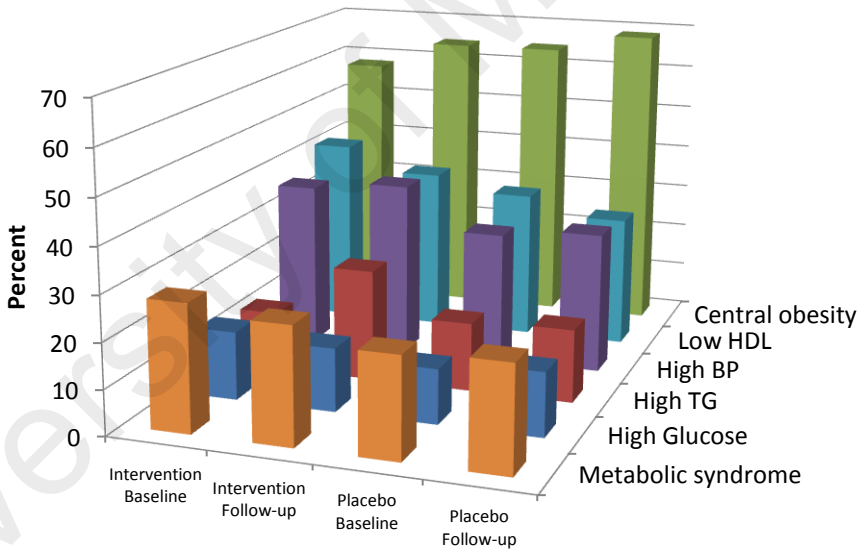

Figure 4.15: Metabolic syndrome and its risk factors among the intervention and placebo groups at baseline and follow-up at 12 months

4.3.5 Health-related quality of life (HRQOL)

The mean scores of all of the domains in SF-36v2 were between 60 and 80 (Table 4.13). In the physical component domain, after 12 months of the intervention, the physical functioning and physical role functioning domains seemed to be slightly reduced in the intervention group (mean difference: -2.835, 95% CI: -9.327 to 3.657 and mean difference: -0.772, 95% CI: -7.287 to 5.743, respectively). In the placebo group, these domains were slightly improved (mean difference: 0.484, 95% CI: -5.672 to 6.640 and mean difference: 0.446, 95% CI: -5.816 to 6.708, respectively), However, there were no significant differences within or between the groups.

Table 4.13: Health-related quality of life parameters in the intervention and placebo groups at baseline and at the 12-month follow-up

	Intervention (n = 93)	Placebo (n = 99)	Mean difference (95% CI) between groups[a]
	Mean (95% CI)	Mean (95% CI)	
Physical functioning			
Baseline	74.68 (70.81 to 78.84)	70.96 (66.13 to 75.79)	3.718 (-2.624 to 10.06)
12 months	71.81 (66.53 to 77.09)	71.48 (66.62 to 76.33)	0.330 (-6.792 to 7.452)
Role physical			
Baseline	74.36 (69.62 to 79.09)	72.27(67.41 to 77.14)	2.082 (-4.662 to 8.826)
12 months	73.45 (67.94 to 78.95)	72.84 (67.78 to 77.90)	0.605 (-6.820 – 8.029)
Bodily pain			
Baseline	73.13 (69.09 to 77.17)	71.13 (67.48 to 74.78)	1.998 (-3.416 to 7.411)
12 months	72.93 (67.76 to 77.09)	69.31 (65.64 to 72.97)	3.621 (-1.891 to 9.132)
General health			
Baseline	66.77 (63.29 to 70.25)	66.12 (62.71 to 69.54)	0.653 (-4.188 to 5.494)
12 months	70.42 (66.85 to 73.99)	68.66 (65.57 to 71.75)	1.763 (-2.924 to 6.449)
Vitality			
Baseline	65.27 (62.80 to 67.74)	61.76(58.99 to 64.53)	3.511 (-0.173 to 7.196)
12 months	65.53 (62.45 to 68.61)	60.49 (57.39 to 63.58)	**5.041 (0.709 to 9.374)***
Social functioning			
Baseline	78.77 (74.76 to 82.79)	74.26 (69.91 to 78.62)	4.512 (-1.377 to 10.401)
12 months	80.27 (76.24 to 84.29)	75.11 (70.66 to 79.56)	5.151 (-0.804 to 11.107)
Role emotional			
Baseline	78.93 (74.32 to 85.53)	76.26 (71.48 to 79.62)	2.662 (-3.938 to 9.262)
12 months	80.52 (75.45 to 85.58)	74.52 (69.43 to 79.62)	5.995 (-1.138 to 13.129)
Mental health			
Baseline	73.12 (70.63 to 75.61)	71.06 (67.74 to 74.37)	2.058 (-2.065 to 6.180)
12 months	75.12 (72.16 to 78.08)	71.53 (68.25 to 74.82)	3.586 (-0.806 to 7.979)
PCS			
Baseline	49.57 (48.17 to 50.97)	48.80 (47.32 to 50.28)	0.772 (-1.249 to 2.793)
12 months	48.99 (47.51 to 50.47)	49.06 (47.79 to 50.32)	-0.069 (-1.997 to 1.860)
MCS			
Baseline	50.09 (48.71 to 51.46)	48.65 (46.82 to 50.48)	1.440 (-0.835 to 3.715)
12 months	51.34 (49.81 to 52.86)	48.39 (46.54 to 50.23)	**2.951 (0.573 to 5.329)***

* Significant at $p < 0.05$ between treatment group
[a] Determined using linear mixed effect model

Both treatment groups appeared to have improved general health, although the participants in the intervention group significantly improved more than those in the placebo group (mean difference: 3.894, 95% CI: 0.057 to 7.730 and mean difference: 2.500, 95% CI: -1.671 to 6.671, respectively). However, there was no significant difference compared

to the placebo group. Although there was an insignificant improvement in vitality in the intervention group (mean difference: 0.249, 95% CI: -3.529 to 4.028) and an insignificant deterioration of vitality in the placebo group (mean difference: -1.197, 95% CI: -5.107 to 2.713), there was a small but significant difference in vitality between the groups. These changes however were clinically not significant. The overall physical component scores showed a deterioration in the intervention group after 12 months of treatment (mean difference: -0.468, 95% CI: -2.158 to 1.222), whereas there was some improvement in the placebo group (mean difference: 0.233, 95% CI: -1.500 to 1.966). However, these differences were not significant within or between the treatment groups.

In the social component domains, the mean scores for social functioning, emotional role functioning and mental health domains were slightly improved in the intervention group after 12 months of treatment; however, there were no significant differences between the treatment groups. In the placebo group, the mean score for the emotional role functioning domain deteriorated slightly, whereas the social functioning and mental health domains were improved. However, there were no significant differences within each treatment group or between the treatment groups. However, the overall mental component scores were significantly different between the treatment groups after 12 months of treatment (mean difference: 2.951, 95% CI: 0.573 to 5.329). There was an insignificant improvement in the intervention group (mean difference: 1.195, 95% CI: -0.387 to 2.776), whereas the placebo group showed some deterioration in the mental component score (mean difference: -.0241, 95% CI: -2.695 to 2.214). There was a significant difference between the groups over time but clinically, these changes were not significant.

4.3.6 Lifestyles practices

4.3.6.1 Dietary intake

Table 4.14 shows that the total caloric intake of the intervention group was slightly higher than that of the placebo group at the 12-month follow-up. The total protein, carbohydrate and fat intakes were also higher in the intervention group compared to the placebo group. None of the above differences were significant ($p > 0.05$). The vitamin D intake was lower at 12 months for both groups. Similar trends were also observed in calcium intake. The differences were not significant within or between the treatment groups.

Table 4.14: 7-day dietary intake of the intervention and placebo groups at baseline and 12-month follow-up

	Intervention (n = 93) Mean (95% CI)	Placebo (n = 99) Mean (95% CI)	Mean difference (95% CI) between group[a]
Energy (kcal/d)			
Baseline	1638.4 (1556.9 to 1719.8)	1680.1 (1582.3 to 1777.5)	-41.7 (-167.9 to 84.4)
12 months	1607.5 (1435.1 to 1779.9)	1556.1 (1474.1 to 1638.2)	51.4 (-138.7 to 241.4)
Protein (g/d)			
Baseline	61.5 (58.6 to 64.4)	64.1 (60.1 to 68.1)	-2.6 (-7.6 to 2.3)
12 months	64.9 (551.9 to 77.8)	60.5 (56.8 to 64.3)	4.4 (-9.1 to 17.8)
Carbohydrate (g/d)			
Baseline	209.7 (198.9 to 220.4)	217.5 (203.1 to 231.8)	-7.83 (-25.66 to 9.99)
12 months	201.4 (190.3 to 212.4)	200.07 (189.6 to 210.6)	1.30 (-13.81 to 16.42)
Fat (g/d)			
Baseline	61.5 (57.3 to 65.6)	61.3 (57.3 to 65.3)	0.2 (-5.5 to 5.9)
12 months	59.9 (49.5 to 70.3)	58.8 (54.2 to 63.4)	1.1 (-10.2 to 12.4)
Vitamin D (IU/d)			
Baseline	253.7 (153.2 to 354.2)	212.4 (136.4 to 288.4)	41.3 (-84.0 to 166.6)
12 months	171.1 (150.1 to 192.0)	189.9 (118.9 to 260.9)	-18.9 (-92.8 to 55.1)
Calcium (mg/d)			
Baseline	420.2 (378.8 to 461.6)	423.5 (384.9 to 462.2)	-3.4 (-59.6 to 52.9)
12 months	395.7 (349.4 to 441.9)	382.2 (350.6 to 412.9)	13.4 (-42.4 to 69.2)
Protein (%/d)			
Baseline	15.3 (14.7 to 15.8)	15.4 (14.9 to 15.9)	-0.2 (-0.9 to 0.6)
12 months	15.6 (14.9 to 16.3)	15.5 (14.9 to 15.9)	0.2 (-0.7 to 0.9)
Carbohydrate (%/d)			
Baseline	51.1 (49.8 to 52.3)	51.6 (50.5 to 52.7)	-0.5 (-2.2 to 1.2)
12 months	51.9 (50.3 to 53.7)	50.9 (49.6 to 52.3)	1.0 (-1.1 to 3.1)
Fat (%/d)			
Baseline	33.3 (32.4 to 34.3)	33.0 (32.1 to 33.9)	0.3 (-1.1 to 1.7)
12 months	32.0 (30.8 to 33.2)	33.33 (32.3 to 34.4)	-1.3 (-2.9 to 0.3)

* Significant at $p < 0.05$ between treatment group

[a] Determined using linear mixed effect model

4.3.6.2 Physical activity (IPAQ scores)

There was an insignificant increase in MET-minutes per week in all physical activity domains in the intervention group after 12 months of follow-up (Table 4.15). In the placebo group, only the leisure-time domains were found to have some increase after 12 months of follow-up, but the increase was not significant ($p > 0.05$). Other domains, such as the work-related, transport-related and domestic and gardening (yard) physical activity domains, showed a reduction after 12 months of follow-up, but it was not significant. Nevertheless, there were no significant differences between the treatment groups for any of the domains. On average, participants from both treatment groups spent at least 300 minutes per day sitting (not included time spent sitting during travel). There was no difference in sitting domain scores between the treatment groups post-intervention ($p > 0.05$).

Table 4.15: IPAQ domain scores of the intervention and placebo groups at baseline and the 12-month follow-up

PA measures (MET-min/week)	Intervention (n = 93) Mean (95% CI)	Placebo (n = 99) Mean (95% CI)	Mean difference (95% CI) between groups[a]
Work			
Baseline	3295.7 (2188.3 to 4403.1)	2706.9 (1713.1 to 3700.8)	588.8 (-889.7 to 2067.3)
12 months	3346.1 (2120.0 to 4572.3)	2177.8 (1293.8 to 3061.9)	1168.3 (-334.6 to 2671.1)
Transportation			
Baseline	580.3 (254.7 to 905.8)	655.8 (325.4 to 986.2)	-75.6 (-536.4 to 385.3)
12 months	1020.5 (454.5 to 1586.6)	483.3 (224.7 to 741.9)	537.2 (-82.8 to 1157.3)
Domestic / garden			
Baseline	1238.3 (959.9 to 1516.5)	1334.3 (1025.9 to 1642.6)	-96.0 (-508.8 to 316.7)
12 months	1563.7 (1069.9 to 2057.4)	1143.9 (881.9 to 1405.8)	419.8 (-136.6 to 976.2)
Leisure-time			
Baseline	378.0 (206.1 to 549.9)	544.7 (257.1 to 832.2)	-166.7 (-500.0 to 166.7)
12 months	397.6 (196.7 to 598.5)	563.3 (161.6 to 965.0)	-165.7 (-612.9 to 281.6)
Sitting*			
Baseline	338.0 (311.9 to 364.2)	323.7 (297.4 to 349.9)	14.4 (-22.5 to 51.2)
12 months	340.0 (313.9 to 366.2)	323.7 (298.7 to 348.6)	16.4 (-19.5 to 52.3)

PA: Physical activities
*minutes/day
[a] Determined using linear mixed effect model

Table 4.16 shows the IPAQ physical activity scores at baseline and at the 12-month follow-up for both treatment groups. In the intervention group, although the total scores for walking and moderate physical activity showed some improvement after 12 months (mean difference: 601.8, 95% CI: -144.9 to 1348.6 MET-min per week; mean difference: 321.1, 95% CI: -372.9 to 1015.9 MET-min per week, respectively), the total vigorous physical activity scores decreased (mean difference: -226.7, 95% CI: -801.6 to 348.2 MET-min per week); however, the changes were not significant ($p > 0.05$). In the placebo group, all of the physical activity scores were found to be decreased after 12 months, but the changes was not significant. There was a between-group difference in moderate physical activity (mean difference: 797.2, 95% CI: 12.8 to 1581.5 MET-min per week), with the intervention group having higher scores. The walking and vigorous physical activity scores were not significantly different between the treatment groups. The overall total physical activity score was decreased in the placebo group after 12 months (mean difference: -806.3, 95% CI: -1797.9 to 185.4 MET-min per week), whereas it was increased in the intervention group (mean difference: 696.2, 95% CI: -674.7 to 2067.2 MET-min per week). However, there was no significant difference between the groups.

Table 4.16: IPAQ physical activity scores of the intervention and placebo groups at baseline and the 12-month follow-up

PA measures (MET-min/week)	Intervention (n = 93) Mean (95% CI)	Placebo (n = 99) Mean (95% CI)	Mean difference (95% CI) between groups[a]
Walking PA			
Baseline	1515.7 (930.6 to 2100.8)	1702.2 (1072.5 to 2331.9)	-186.5 (-1040.6 to 667.6)
12 months	2117.5 (1341.2 to 2893.8)	1536.3 (928.3 to 2144.2)	581.2 (-398.9 to 1561.3)
Moderate PA			
Baseline	1956.1 (1367.9 to 2544.2)	1921.2 (1332.6 to 2509.8)	34.9 (-791.8 to 861.6)
12 months	2277.2 (1610.2 to 2944.2)	1480.0 (1059.7 to 1900.3)	**797.2 (12.8 to 1581.5)***
Vigorous PA			
Baseline	1806.5 (1219.9 to 2392.9)	1332.5 (858.8 to 1806.2)	473.9 (-275.3 to 1223.2)
12 months	1579.8 (913.4 to 2246.1)	1133.3 (680.1 to 1586.5)	446.5 (-354.9 to 1247.8)
Total PA			
Baseline	5278.2 (3730.1 to 6826.3)	4955.9 (3515.2 to 6396.6)	322.3 (-1778.9 to 2423.6)
12 months	5974.5 (4223.1 to 7725.9)	4149.6 (2918.6 to 5380.6)	1824.9 (-303.7 to 3953.4)

PA: Physical activities

* Significant at $p < 0.05$ between treatment group

[a] Determined using linear mixed effect model

When the physical activity scores were classified into three categories (low, moderate and high), more than half of the participants from both groups had moderate physical activity at baseline and after 12 months of follow-up (Table 4.17). However, no differences were observed between the groups ($p > 0.05$).

Table 4.17: Physical activity category scores of the intervention and placebo groups at baseline and the 12-month follow-up

PA Category	Intervention (n = 93)	Placebo (n = 99)	p-value
	n (%)	n (%)	
Low			
Baseline	15 (16.1%)	21 (21.2%)	0.822
12 months	19 (20.4%)	24 (24.2%)	
Moderate			
Baseline	50 (53.8%)	54 (54.5%)	0.832
12 months	53 (57.0%)	54 (54.5%)	
High			
Baseline	28 (30.1%)	24 (24.2%)	0.711
12 months	21 (22.6%)	21 (21.2%)	

4.3.6.3 Sunlight exposure

The participants in the intervention and placebo groups spent more than 50 minutes per week in the sun (mean: 52.17, 95% CI: 35.59 to 68.74 minutes per week and mean: 50.51, 95% CI: 31.34 to 69.69 minutes per week, respectively) (Table 4.18). The mean sun protection score was between three and four for both groups. There were no significant differences within or between the groups.

Table 4.18: Sunlight exposure and sunlight protection scores of the intervention and placebo groups at baseline and the 12-month follow-up

	Intervention (n = 93)	Placebo (n = 99)	Mean difference (95% CI) between groups[a]
	Mean (95% CI)	Mean (95% CI)	
Sunlight exposure*			
Baseline	47.58 (32.44 to 62.72)	43.38 (26.36 to 60.41)	4.19 (-18.44 to 26.84)
12 months	52.17 (35.59 to 68.74)	50.51 (31.34 to 69.69)	1.66 (-23.51 to 26.82)
Sunlight protection			
Baseline	3.08 (2.85 to 3.29)	3.10 (2.91 to 3.29)	-0.03 (-0.32 to 0.27)
12 months	3.09 (2.88 to 3.31)	3.17 (2.95 to 3.39)	-0.07 (-0.37 to 0.23)

*Minutes per week

[a] Determined using linear mixed effect model

Figure 4.16 shows the clothing styles and use of sun protection according to group. Both groups were found to have most of their body covered. Both pre- and post-treatment, most participants in both groups reported wearing clothing styles that covered more than 90% of their body, such asa veil, hijab, long pants or long-sleeved blouses.

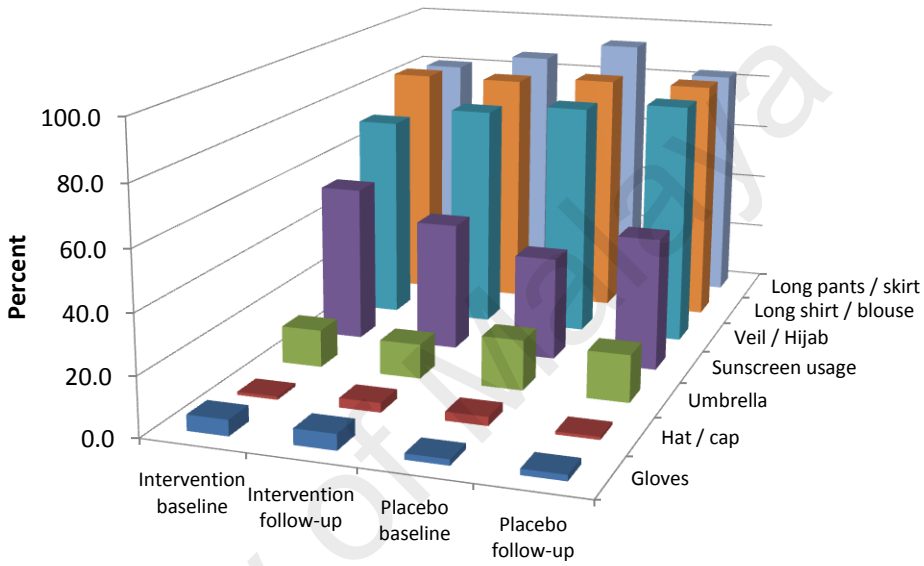

Figure 4.16: Clothing style and sun protection use by treatment groups at baseline and the 12-month follow-up

4.3.7 Sensitivity analysis

Sensitivity analyses using complete cases are presented in Tables 4.19 and 4.20. The results of the sensitivity analyses were consistent with the primary analysis; there were reasonably similar estimates of the treatment effect on the HRQOL parameters, except for the vitality domain. There was difference between the groups at baseline (mean difference: 4.20, 95% CI: 0.23 to 8.18); however, this difference was clinically insignificant.

Table 4.19: Sensitivity analysis using complete cases of outcome measurements in the intervention and placebo groups overtime (baseline, 6 months and 12 months)

	Intervention (n = 83)	Placebo (n = 88)	Mean difference (95% CI) between groups[a]
	Mean (95% CI)	Mean (95% CI)	
25(OH)D (nmol/l)			
Baseline	30.43 (28.54 to 32.32)	30.17 (28.13 to 32.22)	0.26 (-2.51 to 3.02)
6 month	83.88 (79.46 to 88.30)	37.41 (34.89 to 39.94)	**46.47 (41.40 to 51.53)***
12 months	85.77 (80.81 to 90.72)	36.23 (33.57 to 38.88)	**49.54 (43.94 to 55.14)***
PTH (pmol/l)			
Baseline	4.51 (4.03 to 4.98)	4.76 (4.36 to 5.15)	-0.25 (-0.86 to 0.36)
6 month	4.49 (4.04 to 4.96)	5.81 (5.27 to 6.34)	**-1.31 (-2.01 to -0.61)***
12 months	4.19 (3.69 to 4.71)	5.22 (4.81 to 5.63)	**-1.02 (-1.67 to -0.37)***
Calcium (mmol/l)			
Baseline	2.19 (2.17 to 2.20)	2.19 (2.17 to 2.20)	-0.002 (-0.023 to 0.020)
6 month	2.23 (2.20 to 2.26)	2.24 (2.22 to 2.25)	-0.004 (-0.038 to 0.031)
12 months	2.24 (2.22 to 2.26)	2.22 (2.20 to 2.24)	0.017 ((-0.011 to 0.046)
HOMA-IR			
Baseline	3.90 (2.01 to 5.79)	2.53 (2.43 to 2.66)	1.37 (-0.79 to 3.53
6 month	3.06 (2.51 to 3.61)	2.84 (2.30 to 3.41)	0.21 (-0.57 to 0.98)
12 months	3.19 (2.61 to 3.78)	2.99 (2.41 to 3.56)	0.21 (-0.61 to 1.03)
LDL (mmol/l)			
Baseline	3.29 (3.13 to 3.47)	3.29 (3.15 to 3.45)	-0.001 (-0.226 to 0.223)
6 month	3.25 (3.09 to 3.42)	3.31 (3.17 to 3.46)	-0.059 (-0.277 to 0.160)
12 months	3.62 (3.42 to 3.81)	3.63 (3.47 to 3.78)	-0.013 (-0.261 to 0.236)
HDL (mmol/l)			
Baseline	1.45 (1.34 to 1.56	1.45 (1.38 to 1.53)	0.00 (-0.14 to 0.13)
6 month	1.44 (1.37 to 1.52)	1.50 (1.44 to 1.57)	-0.06 (-0.16 to 0.04)
12 months	1.52 (1.44 to 1.60)	1.50 (1.43 to 1.57)	0.02 (-0.09 to 0.13)
TG (mmol/l)			
Baseline	1.20 (1.08 to 1.31)	1.19 (1.05 to 1.32)	0.012 (-0.162 to 0.187)
6 month	1.39 (1.24 to 1.55	1.20 (1.09 to 1.31)	**0.192 (0.006 to 0.378)***
12 months	1.36 (1.21 to 1.51)	1.22 (1.10 to 1.34)	0.14 (-0.049 to 0.329)
SBP			
Baseline	122.3 (118.9 to 125.8)	119.3 (116.2 to 122.4)	3.07 (-1.53 to 7.66)
6 month	126.7 (123.0 to 130.5)	123.9 (120.9 to 127.0)	2.79 (-1.98 to 7.57)
12 months	125.8 (122.6 to 128.9)	123.9 (120.7 to 127.0)	1.89 (-2.55 to 6.34)
DBP			
Baseline	78.07 (75.83 to 80.31)	76.88 (74.58 to 78.17)	1.197 (-1.987 to 4.382)
6 month	79.83 (77.34 to 82.33)	79.27 (77.01 to 81.54)	0.559 (-2.788 to 3.905)
12 months	77.52 (75.15 to 79.89)	76.76 (74.57 to 78.95)	0.757 (-2.446 to 3.959)

* Significant at $p < 0.05$ between groups

[a] Determined using linear mixed effect model

Table 4.20: Sensitivity analysis using complete cases of health-related quality of life parameters in the intervention and placebo groups overtime (baseline and 12 months)

	Intervention (n = 83)	Placebo (n = 88)	Mean difference (95% CI) between groups[a]
	Mean (95% CI)	Mean (95% CI)	
Physical functioning			
Baseline	74.28 (60.81 to 78.75)	71.14 (65.96 to 76.32)	3.14 (-3.65 to 9.94)
12 months	71.81 (66.53 to 77.09)	71.48 (66.62 to 76.33)	0.33 (-6.79 to 7.45)
Role physical			
Baseline	73.75 (68.79 to 78.70)	72.85(67.79 to 77.91)	0.90 (-6.14 to 7.93)
12 months	73.4567.94 to 78.95)	72.84(67.78 to 77.90)	0.6 (-6.82 – 8.03)
Bodily pain			
Baseline	72.39 (68.11 to 76.66)	71.09 (67.36 to 74.83)	1.30 (-4.34 to 6.93)
12 months	72.93 (68.76 to 77.10)	69.31 (65.64 to 72.97)	3.62 (-1.89 to 9.13)
General health			
Baseline	66.17 (62.45 to 69.89)	66.35 (62.87 to 69.84)	-0.18 (5.24 to 4.88
12 months	70.42 (66.85 to 73.99)	68.66 (65.57 to 71.75)	1.76 (-2.92 to 6.45)
Vitality			
Baseline	65.39 (62.72 to 68.05)	61.18(58.19 to 64.17)	**4.20 (0.23 to 8.18)***
12 months	65.53 (62.45 to 68.61)	60.49 (57.39 to 63.58)	**5.04 (0.71 to 9.37)***
Social functioning			
Baseline	78.31 (74.05 to 82.58)	74.47 (69.79 to 79.14)	3.85 (-2.44 to 10.13)
12 months	80.27 (76.24 to 84.29)	75.11 (70.66 to 79.56)	5.15 (-0.80 to 11.11)
Role emotional			
Baseline	78.70 (73.73 to 83.67)	76.14 (70.99 to 81.29)	2.56 (-4.55 to 9.67)
12 months	80.52 (75.45 to 85.58)	74.52 (69.43 to 79.62)	5.99 (-1.14 to 13.13)
Mental health			
Baseline	73.61 (71.02 to 76.21)	70.97 (67.39 to 74.54)	2.65 (-1.74 to 7.04)
12 months	75.12 (72.16 to 78.08)	71.53 (68.25 to 74.82)	3.59 (-0.81 to 7.98)
PCS			
Baseline	49.22 (47.76 to 50.67)	48.94 (47.40 to 50.49)	0.27 (-1.83 to 2.38)
12 months	48.99 (47.51 to 50.47)	49.06 (47.79 to 50.32)	-0.07 (-1.99 to 1.86)
MCS			
Baseline	50.22 (48.71 to 51.72)	48.47 (46.47 to 50.46)	1.75 (-0.73 to 4.23)
12 months	51.34 (49.81 to 52.86)	48.39 (46.54 to 50.23)	**2.95 (0.57 to 5.33)***

* Significant at $p < 0.05$ between groups

[a] Determined using linear mixed effect model

4.4 Summary of results

4.4.1 Baseline characteristics

In summary, the participants in both groups were found to be comparable in terms of socio-demographic characteristics at baseline. The majority of the participants were Malays, ranged in age from 40 to 45 years and had at least a secondary level of education. Out of 369 premenopausal women aged 30 to 55 years who underwent serum 25(OH)D analysis, 344 (93.2%) were found to be vitamin D deficient. However, only 192 women agreed to participate in this study, and 171 completed the 12-month follow-up.

At baseline, history of chronic diseases and metabolic syndrome and its characteristics were comparable in both groups. The dietary intakes of both groups were comparable. The physical activity of both groups was largely related to domestic duties, gardening and work; there was less leisure-time physical activity. Less-vigorous physical activity was also observed in the participants from both treatment groups. However, the difference between the groups was not significant. Participants in both groups were also found to spend more time outdoors exposed to sunlight. At baseline, all of the clinical, biochemical and anthropometric measurements were comparable between the groups. Similarly, the HRQOL scores of both groups were comparable.

4.4.2 Effects of vitamin D supplements on serum vitamin D, PTH and calcium levels

Following the intervention programme, the intervention group showed a significant improvement in serum 25(OH)D over 12 months ($p < 0.001$). There was also a significant increase in mean serum 25(OH)D in the placebo group; however, the level was still in the deficient category (<50 nmol/l). A total of 91.6% of the intervention group achieved a 25(OH)D level greater than 50 nmol/l (20 ng/ml), and 63.4% achieved a level of 75 nmol/l

(30 ng/ml) or higher. In contrast, 13.1% of the placebo group had a 25(OH)D level above 50 nmol/l, and only 1% had a value of 75 nmol/l or higher. The mean PTH concentration remained suppressed in the intervention group as the mean serum 25(OH)D increased; however, the change in PTH was not significant. In the placebo group, the mean PTH concentration increased from baseline to six months but declined slightly at 12 months. There were significant differences in the mean concentrations of total 25(OH)D and PTH between the groups after 12 months of treatment. There was a significant difference in serum calcium within the intervention group between baseline and six months. However, after six months, the serum calcium level remained stable without any significant increase.

4.4.3 Effects of vitamin D supplements on cardiometabolic risk factors

There was no improvement in either indicator of obesity (BMI and WC) in either group between baseline, six months and 12 months. There were significant changes in LDL within the intervention group from baseline to 12-months ($p < 0.05$), but the changes were clinically insignificant. Similarly, there were statistically significant but clinically insignificant changes in mean serum calcium and LDL over time within the placebo group ($p < 0.05$). These changes, however, did not differ between the groups and were clinically insignificant. An increase in serum 25(OH)D in the intervention group induced a reduction in HOMA-IR, whereas the reverse was observed in the placebo group. However, these changes were not significantly different between the groups. A sensitivity analysis using complete cases showed similar results.

4.4.4 Effects of vitamin D supplements on HRQOL

At baseline, the mean scores on all of the HRQOL components of the SF-36 ranged from 60 to 70. Vitamin D supplementation improved the general health of the intervention group. However, there was no significant difference compared to the placebo group. There

were no improvements in the HRQOL components between groups over time. Sensitivity analyses using complete cases did not differ from the primary analyses. Although the HRQOL vitality domain was found to be significantly different at baseline, this difference was clinically insignificant.

4.4.5 Lifestyle practices

Dietary intake, physical activity, sunlight exposure and sunlight protection did not change between baseline and 12 months of follow-up for either the intervention or placebo groups. Moderate physical activity was found to be significantly different between the groups after 12 months of treatment; however, the total physical activity of both groups was not significantly different.

CHAPTER 5 : DISCUSSION

5.1 Baseline characteristics of participants

5.1.1 Socio-demographic characteristics of participants

The participants in both groups had a similar background in terms of age, race and level of education. A majority of the participants in the intervention and placebo groups were Malays and Indians. However, the distribution of ethnicity in this setting did not reflect the national distribution of ethnic groups in the Malaysian population, of which Malays constitute 63.1%, followed by Chinese (24.6%), Indians (7.5%) and others (4.8%) (Department of Statistics, 2010). The reason for discrepancy this might be that more Malays prefer to work in the government sector (UM is a public university), whereas the Chinese prefer to work in the private sector.

Most of our participants had at least a secondary education. The Malay participants were Muslim by religion; most of them wore a hijab and covered almost 90% of their body surface, as required by their religion. The Malays and Indians also tend to have darker skin pigmentation.

The majority of our participants worked as administrative workers (e.g., clerks or secretaries) or lecturers. They worked indoors most of the time. The participants employed as general workers (gardeners or cleaners) spent most of their working hours outside the building, gardening or cleaning the building's premises.

5.1.2 History of chronic diseases

The prevalence of chronic diseases reported by the participants in both groups was comparable. Although they were vitamin D deficient, the proportion of participants suffering from chronic diseases, such as hypertension or diabetes, was relatively low

compared to the general population. The National Health and Morbidity Survey IV (NHMS IV) conducted in 2011 found that only 14.5% of women aged more than 18 years were diabetic, whereas 31.6% were hypertensive (Ministry of Health, 2011). Similarly, less than approximately 30% of our participants had metabolic syndrome, which was lower than the general population (42.5%) (Mohamud et al., 2011). The risk of metabolic syndrome is known to be closely linked to being overweight, obesity, physical inactivity and insulin resistance. Because more than 70% of our participants were overweight or obese and more than 50% of them had insulin resistance, our participants were at risk of metabolic syndrome and cardiovascular diseases, such as myocardial infarction or stroke, in the future. Individuals with metabolic syndrome have a higher chance of developing cardiovascular diseases (Mottillo et al., 2010; Wilson, D'Agostino, Parise, Sullivan, & Meigs, 2005). In another study, Isomaa et al. (2001), found that subjects with metabolic syndrome had a three-fold higher risk of coronary heart disease and stroke compared to those without metabolic syndrome.

5.1.3 Anthropometric, clinical and biochemical measurements

The mean BMIs of the participants in both groups were comparable and were in the overweight category. In the NHMS IV report, the prevalence of overweight Malaysians aged between 30 and 50 years was 35.4% to 41.0%; in contrast, more than 70% of our participants were overweight.

The mean SBP, DBP, HDL, and TG values at baseline for both groups were within the normal range, with the majority being normal. Similarly, the plasma glucose levels were also within the normal range. However, the mean LDL was above the normal range for both groups, with the majority of participants having abnormal rates (>70%). More than 50% of the participants in both the intervention and placebo groups had abnormal HOMA-

IR, although the median glucose level was within the normal range. Nevertheless, all of the clinical and biochemical measurements were comparable between the groups.

5.1.4 Health-related quality of life (HRQOL)

All of the components of HRQOL were found to be comparable between the groups. The scores of participants in both groups on some of the HRQOL components, namely physical functioning (intervention: 74.68; placebo: 70.96), physical role functioning (intervention: 74.35; placebo: 72.27) and social functioning (intervention: 78.77; placebo: 74.26) were lower than those of the general population (84.52, 81.47 and 82.94, respectively) (Azman et al., 2003).

Regarding the bodily pain component, the general population perceived more bodily pain (68.96) than our participants (intervention: 73.13; placebo: 71.13). This finding was not expected because muscle and bone pain are common symptoms of vitamin D deficiency, which can affect the physical functioning and physical role functioning components of HRQOL. However, the participants' general health, vitality, emotional role functioning and mental health component summary scores were found to be similar to those of the general population.

Vitamin D is a nuclear steroid hormone thought to be involved in brain health and function as well as neuromuscular functions. When bound to 1,25(OH)D, vitamin D receptors in the cell's nucleus regulate the expression of target genes. These receptors are expressed in areas of the brain important for behavioural regulation (Kalueff & Tuohimaa, 2007). Therefore, vitamin D may affect the mental components of people with vitamin D deficiency.

5.1.5 Lifestyle practices

5.1.5.1 Dietary intake

A twenty-four-hour, seven-day dietary record was used to assess the participants' daily dietary intake. The vitamin D intakes of both groups were comparable. However, their median vitamin D intake did not meet the Malaysian RNI. The recommended vitamin D intake for Malaysian women aged 30 to 50 years is 5 µg/ day (200 IU/day) (Nutrition, 2005). Only 23.7% and 29.3% of the participants in the intervention and placebo groups, respectively, met the RNI for vitamin D intake. To our knowledge, there has been no nationwide study on vitamin D nutrient intake in Malaysia to date. Only pockets of small studies were available that reported vitamin D nutrient intake. A study on vitamin D status in postmenopausal Malaysians reported that the mean vitamin D intake was 9.1 ± 12 µg/day in Malays and 8.4 ± 19 µg/day in Chinese (Rahman et al., 2004). Another study reported that the daily vitamin D intake in adolescents in Malaysia was 0.6 µg/day (95% CI: 0.5 to 0.7µg/day) (Hazreen AM, 2014). Nurbazlin et al. (2013) reported that the median vitamin D intake in urban women older than 45 years was 4.61 (Q1-Q2: 2.66 to 7.41) µg per day. They also found that only 7.5% of urban adults met the vitamin D RNI, which was slightly higher than our findings.

The total energy intakes for both groups were slightly higher than the general population, which the Malaysian Adults Nutrition Survey (MANS) reported was 1400 kcal for women (Mirnalini et al., 2008). Similarly, the MANS study reported total carbohydrate, protein and fat intakes that were also lower than those of our participants. The daily calcium intakes of both groups were lower than the Malaysian RNI (intervention: 420.2 ± 200.9 mg/d; placebo: 423.5 ± 193.9 mg/d). The Malaysian RNI for calcium for women aged >18 years is more than 800 mg/day (Nutrition, 2005).

5.1.5.2 Physical activity

Overall, the IPAQ scores were comparable between the groups. The participants in this study engaged in domestic chores more than other domains of physical activity, which was followed by occupation-related physical activity and active transportation. Leisure-time physical activities were the least common type of physical activity engaged in by our participants. This finding was expected because the participants spent most of their time at work during the daytime, and their leisure time was usually filled with domestic chores, such as sweeping and cleaning the home. Therefore, they hardly had time for leisure-time physical activities, such as jogging or sports activities, even during the weekend. A majority of the participants worked as administrative workers, such as clerks or secretaries, which required them to perform desk work. Therefore, the median total walking scores were low for both groups (intervention group: 495, IQR: 1914 MET-min per week; placebo group: 429, IQR: 1221 MET-min per week), and the median sitting scores were high (intervention group: 342.9, IQR: 215.7 minutes per day; placebo group: 328.6, IQR: 202.9 minutes per day).

Similar findings were reported by A. H. Y. Chu and Moy (2014) who studied a population in the same setting as this study, and in a report on physical activity patterns in Malaysian adults that concluded that Malaysian adults were generally sedentary (Poh et al., 2010). Adopting a healthy lifestyle, such as an active lifestyle and a healthy diet, can prevent or delay the development of metabolic syndrome and its risk factors (A. H. Y. Chu & Moy, 2014; Gaesser, Angadi, & Sawyer, 2011; Shrestha & Ghimire, 2012; Volpe et al., 2012).

5.1.5.3 Sunlight exposure

The participants in our study spent at least 40 minutes per week exposed to sunlight. The mean sunlight exposure scores were comparable between the groups. The time spent outdoors exposed to sunlight was expected to be low in our participants because the majority worked indoors most of the day. Their working hours were between 7.30 am and 5.30 pm. Among the participants who worked as general workers, a majority of their time was spent outdoors; they were in the sun at least 60% of their working hours.

A study by Islam et al. (2010) reported that being exposed to the sun at least 20 to 45 minutes per week would result in sufficient production of vitamin D by the skin. Similar findings were reported by Dowdy et al. (2010). Nevertheless, our participants from both groups were vitamin D deficient despite reporting a sunlight exposure score of more than 45 minutes per week. This finding could be due to their clothing styles. Most of the participants covered up more than 90% of their body due to religious and cultural practices. A few studies have shown that females wearing hijab have lower 25(OH)D plasma levels than those wearing Western-style clothing (Mallah et al., 2011).

Another issue that could cause insufficient production of vitamin D by the skin despite being exposed to sunlight is usage of sunscreen. A sunscreen with a sun protection factor of 15 reduces the vitamin D production in the skin by 99% (Matsuoka et al., 1992). However, only 35.4% of the participants in the placebo group and 53.8% of the participants in the intervention group reported wearing sunscreen for sunlight protection.

5.2 Process evaluation

Out of 389 potentially suitable participants for our study, 344 (93.2%) were identified to be vitamin D deficient and eligible for the study. However, out of 344 eligible participants,

only 192 (55.8%) consented to participate. Throughout the study, 171 participants (89%) completed the follow-up, and 21 participants withdrew from the study. The reasons for dropping out were perceived side effects (two participants), pregnancy during the follow-up (eight participants), and being prescribed vitamin D > 1000 IU/day due to bone fractures secondary to a motor vehicle accident (one participant); no reasons were provided by10 participants.

There is a possibility that the dropouts may have biased our results; however, there may be a low bias effect on our results because (a) none of the baseline characteristics were significantly different between the withdrawn and complete case participants and (b) the proportions of withdrawn participants in both groups were comparable. In addition, a linear mixed-effects analysis was used to reduce the bias from missing values arising from the dropouts. We also monitored the participants' compliance during every follow-up. The rates of compliance with the intervention or placebo were excellent.

5.3 Outcome evaluation

5.3.1 Effects of vitamin D supplementation on serum 25(OH)D

There was a significant improvement in the mean serum 25(OH)D level in the intervention group after 12 months of follow-up, especially in the first six months, when the circulating serum 25(OH)D level in the intervention group increased to above 80 nmol/l. However, the level plateaued at 12 months, which could be because the maintenance dosage provided was not sufficient to increase the circulating serum 25(OH)D level to above 80 nmol/l. The treatment regimen provided in the first two months (50,000 IU fortnightly) was sufficient to achieve a sufficient serum 25(OH)D level (> 75 nmol/l); however, the maintenance dosage, which was 50,000 IU per month (equivalent to 1666 IU per day) may not have been sufficient to increase the level to at least 100 nmol/l. Although

the Endocrine Society Clinical Practice Guidelines recommend a maintenance therapy of 1500 to 2000 IU per day after the serum 25(OH)D achieves a sufficient level (75 nmol/l) (Holick et al., 2011), H. Bischoff-Ferrari (2009) suggested that consistently increasing the circulating serum 25(OH)D above 75 nmol/l may require at least 5000 IU/day of vitamin D supplements.

Frequent serum 25(OH)D assessments would be needed to observe when the circulating serum 25(OH)D reached a peak (> 100 nmol/l), which could be less than six months. However, we did not perform such frequent assessments in our study due to budget constraints. Performing frequent assessments of serum 25(OH)D would have also given us information about the interval at which the level started to drop below 100 nmol/l when the maintenance dose was provided. This information would be important to show whether the maintenance dosage provided (50,000 IU per month) was sufficient to maintain the serum 25(OH)D level above 100 nmol/land at what time one should start maintenance therapy. Recent studies have suggested that the serum 25(OH)D level should be at least above 100 nmol/l to achieve the benefits of vitamin D associated with cardiovascular risk factors (Talaei et al., 2013; von Hurst et al., 2010).

More than 90% of the participants in the intervention group achieved serum 25(OH)D above 50 nmol/l, compared to only 13% in the placebo group. These results were comparable to other studies, which used a daily dosage of vitamin D (von Hurst et al., 2010; Wood et al., 2012). The small proportion of participants in the intervention group who did not achieve a sufficient level of circulating vitamin D could be due to obesity. Further exploration of the data revealed that six out of seven of these women were obese (BMI > 25 kg/m^2). The bioavailability of vitamin D sequestered in the fat of individuals with excess adipose tissue is decreased, which might result in a reduced circulating serum 25(OH)D concentration (Kevin C. Maki et al., 2009; Wortsman et al., 2000). Harris et al.

(2012) and Maki et al. (2011) also reported that vitamin D supplementation in predominantly overweight and obese participants was not sufficient to achieve a serum 25(OH)D greater than 75 nmol/l. These findings support the growing evidence that overweight individuals may be at risk of adverse outcomes related to vitamin D deficiency and may need long-term vitamin D supplementation. Ekwaru et al (2014) recommended that obese participants might need a two- to three-fold higher dosage of vitamin D supplementation to maintain a sufficient serum 25(OH)D concentration.

5.3.2 Effects of vitamin D supplementation on serum PTH

Serum PTH was improved in the intervention group after 12 months of treatment, and these changes were significantly different between the groups ($p < 0.001$). The serum PTH level seemed to decrease along with the improvement in the serum 25(OH)D level in the intervention group (>80 nmol/l). In the placebo group, the serum PTH level continued to increase as the serum 25(OH)D remained below sufficiency level (< 50 nmol/l). Similar findings have been reported elsewhere (Jorde et al., 2010; Nagpal et al., 2009; Ryu et al., 2013; Sollid et al., 2014; Yiu et al., 2013). A study by Laufey Steingrimsdottir (2005) concluded that as long as serum 25(OH)D remained at deficiency levels, even high calcium intake (>1200 mg/day) would not be able to maintain the serum PTH at the desired level. The threshold level for PTH elevation is when the serum 25(OH)D level is less than 75 nmol/l (A. Zittermann, 2006). Therefore, the circulating vitamin D concentration should be above 75 nmol/l level to ensure that the serum PTH is maintained at a sufficient level.

Elevated PTH concentrations are known to be associated with cardiometabolic diseases via multiple mechanisms, such as increases in arterial pressure and myocardial contractility, which can lead to apoptosis, fibrosis and vascular smooth muscle cell hypertrophy as well as left ventricular hypertrophy (Lavie, Dinicolantonio, Milani, &

O'Keefe, 2013; A. Zittermann, 2006). In the elderly, secondary hyperparathyroidism was found to be associated with coronary heart disease incidence (Soubassi et al., 2006) and nearly two-fold increase in mortality (Bjorkman, Sorva, & Tilvis, 2008). Therefore, there is a need to ensure vitamin D sufficiency to maintain an ideal serum PTH concentration because hyperparathyroidism is associated with increased risk of cardiometabolic diseases.

5.3.3 Effects of vitamin D supplementation on serum calcium

The mean serum calcium in the intervention group and the placebo group was maintained within the normal limit at baseline and at 12 months of follow-up. There was a small but insignificant increase in serum calcium levels in the intervention group from baseline to 12 months of follow-up as the serum 25(OH)D was increased to a sufficient level. In the placebo group, serum calcium levels were also observed to have some increase in the first six months, but they subsequently declined. Nevertheless, these changes were not significant within or between the groups ($p > 0.05$).

Vitamin D is a regulator of systemic calcium homeostasis, which helps in the absorption of calcium in the intestine, kidney and bone. PTH is a major hormone that facilitates the maintenance of normal serum calcium. In our study, although the serum 25(OH)D concentrations increased to greater than 50 nmol/l with a reduction in serum PTH, only a small but insignificant increase in calcium was observed in the intervention group. A possible explanation for this finding is the low daily calcium intake in our participants, which was lower than the Malaysian RNI (Nutrition, 2005). Ekwaru et al. (2014) found that for every 1000 IU increase in daily vitamin D supplementation, serum calcium only increased by 0.001 mmol/l. Therefore, even with vitamin D supplementation, adequate calcium intake, either via supplements or food, is still needed to maintain an ideal serum

calcium concentration to observe health benefits. A study by Asemi et al. (2014) observed a significant increase in serum calcium in the intervention group given calcium and vitamin D co-supplementation compared to the groups receiving placebo or a single nutrient. Many studies have also used combinations of vitamin D and calcium supplementation to ensure a sufficient intake of both vitamin D and calcium to result in a beneficial effect on cardiometabolic risk (Jorde et al., 2010; Major et al., 2007; Mitri et al., 2011; Pittas, Harris, et al., 2007; Ryu et al., 2013).

5.3.4 Effects of vitamin D on cardiometabolic risk factors

5.3.4.1 Insulin resistance and blood glucose

Type 2 diabetes mellitus is characterized by a relative deficiency of insulin secretion and insulin resistance in peripheral tissues (Lillioja et al., 1993; Martin et al., 1992). Individuals who develop diabetes may manifest primary defects in insulin action and insulin secretion, which predispose them to diabetes mellitus. As discussed in section 2.2.8.1, HOMA-IR is one of the surrogate markers commonly used to gauge insulin resistance. Therefore, interventions for diabetes prevention, especially among people who are at high risk, should begin at an early stage and should target both insulin resistance and insulin secretory dysfunction.

There was a slight increase in the HOMA-IR level from baseline to 12 months of follow-up in the placebo group, whereas a reduction was observed in the intervention group; however, the mean difference was not significant. The HOMA-IR levels of both groups at baseline and after 12 months of follow-up were much higher than the HOMA-IR values reported in another study in a healthy Malay population (HOMA-IR: 1.05 ± 0.96 to 1.13) (A. K. Al-Mahmood, A. A. Ismail, F. A. Rashid, & W. M. Wan Bebakar, 2006). This result showed that our participants were at risk of developing type 2 diabetes mellitus.

Theoretically, vitamin D supplementation should suppress the PTH level, and this mechanism could favourably affect glucose mechanism by enhancing insulin signalling in adipocytes, thus improving insulin resistance. However, we failed to detect any improvement in glucose levels or insulin resistance despite suppressing the PTH level after a year of vitamin D supplementation. Our findings are consistent with a few other studies (Asemi et al., 2014; Breslavsky et al., 2013; Harris et al., 2012; Jorde et al., 2010; Nagpal et al., 2009; Pittas, Harris, et al., 2007; Ryu et al., 2013; Sollid et al., 2014; Strobel et al., 2014; Tai et al., 2008; Witham, Dove, et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009).

A possible explanation for these negative results could be related to not using the optimal dose of vitamin D supplementation for reducing cardiometabolic risk. Expert recommendations for daily vitamin D dosage range from 600 to 2000 IU for the prevention of cardiovascular or metabolic risk (Pittas, Lau, et al., 2007; Ross et al., 2011; Vacek et al., 2012; Whiting, Evans, & Foo, 2013). The Institute of Medicine, on the other hand, has not made any recommendations regarding the optimal dose of vitamin D supplementation in relation to non-skeletal outcomes due to a lack of evidence (Ross et al., 2011). There is a possibility that for the prevention of cardiometabolic risk factors, a dosage of vitamin D supplementation higher than 600 IU per day was needed to increase the level of circulating serum vitamin D at an optimal level (> 100 nmol/l). The current RNI for vitamin D of 600 IU per day may not be sufficient for the prevention of cardiometabolic risk factors and diseases; furthermore, 600 IU/day produces barely perceptible changes in individuals who are overweight or obese.

Another possible reason could be the failure to maintain circulating vitamin D over time. Daily doses of vitamin D supplementation may lead to more stable circulating serum vitamin D compared to longer interval dosing, which may result in larger fluctuations in

serum 25(OH)D (Hollis & Wagner, 2013). As a result, studies that used weekly or monthly doses of vitamin D, similar to our study, obtained negative results (Jorde et al., 2010; Nagpal et al., 2009; Sollid et al., 2014; Tai et al., 2008; Witham, Dove, et al., 2010).

Nevertheless, we found a few studies that also observed negative findings despite giving daily vitamin D supplementation as an intervention (Breslavsky et al., 2013; Harris et al., 2012; Ryu et al., 2013; Strobel et al., 2014; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009). This outcome is possibly due to the serum 25(OH)D concentration at the end of the intervention being less than 85 nmol/l. Problems complying with daily treatment may be another explanation why the serum 25(OH)D concentration was still below 85 nmol/l despite treatment with at least 1000 IU per day. von Hurst et al. (2010) reported that their participants found it difficult to comply with a daily dosage of vitamin D, thus causing their serum 25(OH)D concentration to be below 75 nmol/l despite taking 2000 IU/day of vitamin D supplements.

Contrary to our results, few other clinical trials showed that vitamin D supplementation improved plasma glucose and insulin resistance (Aljabri et al., 2010; Asemi et al., 2014; Mitri et al., 2011; Pittas, Harris, et al., 2007; Talaei et al., 2013; von Hurst et al., 2010). This outcome could be due to an indirect effect of vitamin D on pancreatic β-cells, which mediated via extracellular calcium regulation. This calcium-dependent process affects insulin secretion, thus improving plasma glucose. Therefore, a combination of vitamin D and calcium may be more beneficial in relation to cardiometabolic risk prevention than either nutrient alone (Mitri et al., 2011; Pittas, Harris, et al., 2007). Trials using combinations of vitamin D and calcium (> 500 mg/day) have observed positive results (Aljabri et al., 2010; Asemi et al., 2014; Mitri et al., 2011; Pittas, Harris, et al., 2007) compared to trials using vitamin D supplements alone as the intervention, as in our study (Gepner et al., 2012; Sollid et al., 2014; Tai et al., 2008; Witham, Dove, et al., 2010; Wood

et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009). A prospective study by Pittas et al. (2006) found that a combined daily intake of more than 1200 mg of calcium and more than 800 IU of vitamin D was associated with a 33% lower risk of type 2 diabetes. A systematic review and meta-analysis supported this finding, which concluded that the optimal calcium intake in relation to type 2 diabetes mellitus was greater than 1200 mg/d (Pittas, Lau, et al., 2007). Nevertheless, one study did not find any improvement in HOMA-IR despite giving a combination of vitamin D supplementation and calcium to the participants (Ryu et al., 2013). This finding could be due to the lower dosage of calcium provided (200 mg/day), which was much lower than the recommended daily calcium (> 800 mg/day).

Another explanation for the positive findings of some studies was the selection of subjects with abnormal IR or FBS at baseline. The participants in our study had glucose values in the normal range at baseline, which may be difficult to improve further. A study by Pittas et al. (2007) observed a significant improvement in HOMA-IR in subjects with impaired fasting glucose at baseline, whereas there was no significant difference in HOMA-IR in subjects with normal fasting glucose at baseline despite receiving 500 mg of calcium with 700 IU of vitamin D as the intervention. In the von Hurst et al. (2010) study, although the intervention did not include calcium (4000 IU/day vitamin D), the subjects were vitamin D deficient and insulin resistant at baseline. The same study also demonstrated that to observe a significant improvement in insulin resistance, the mean serum 25(OH)D concentration at the end of the trial must be greater than 85 nmol/l. This finding was also supported by Talaei et al. (2013) who demonstrated that insulin resistance was significantly improved at a serum 25(OH)D concentration between 100 and 150 nmol/l. However, that study did not have a comparison or placebo group. Therefore, we do not know whether the improvement was strictly due to vitamin D supplementation.

Nevertheless, a systematic review and meta-analysis concluded that current evidence is still insufficient to establish a causal relationship between vitamin D supplementation and insulin resistance and glycaemia improvement (George, Pearson, & Witham, 2012). Additional larger-scale clinical trials are needed to support the current evidence using different dosages of vitamin D supplementation and vitamin D co-supplementation with calcium to enhance the beneficial effects on cardiometabolic risk prevention.

5.3.4.2 Low-density lipoprotein (LDL)

Despite the ability of a high dosage of vitamin D supplementation to improve vitamin D status in the intervention group, we found no significant improvement in lipid profiles. In fact, we even observed adverse effects of vitamin D supplementation on LDL. A similar trend was observed in the placebo group. Similar findings were reported by Zitterman et al. (2009) who found a significant 5% LDL increase in the intervention group. A few other studies have observed similar findings (Jorde et al., 2010; K. C. Maki et al., 2011; Wood et al., 2012). Our findings were supported by a meta-analysis by Wang et al. (2012), who concluded that vitamin D supplementation provided a significant increase in LDL (3.23 mg/dl or 0.08 mmol/l).

A possible reason for these negative results is the effect of vitamin D on calcium intestinal absorption. Vitamin D induces the intestinal absorption of calcium, causing a lower calcium content in the gut; thus, insoluble calcium-fatty soap formation is reduced in the gut, resulting in a faecal fatty acid content reduction and thus more lipids in the blood (Reid, 2004; Van der Meer et al., 1990; Vaskonen, 2003). In addition, the increase in intestinal calcium absorption induced by vitamin D decreases calcium bile acid binding, which reduces cholesterol conversion to bile acids and therefore reduces cholesterol

excretion, which consequently increases the absorption of cholesterol into the blood. With adequate intake of calcium and vitamin D, the absorption of calcium was 65% higher at a serum 25(OH)D level greater than 85 nmol/l (Heaney, Dowell, et al., 2003). The remaining calcium in the intestine would participate in calcium fatty soap formation and calcium bile acid binding. Therefore, an adequate intake of both calcium and vitamin D is important to reduce the cholesterol level in the blood. In our study, despite receiving vitamin D supplements, our participants had low dietary calcium intake (intervention group: 420.18 mg/d; placebo group: 423.53 mg/day), which might explain why there was no significant improvement in LDL.

Therefore, it is predictable that studies using combinations of vitamin D and calcium will obtain positive findings in relation to lipid profiles (Asemi et al., 2014; Major et al., 2007). The reason for the co-supplementation of vitamin D and calcium is to ensure that the dietary calcium and vitamin D intakes are adequate. Major et al. (2007) gave 200 IU of vitamin D with 600 mg of calcium supplementation daily to healthy overweight and obese participants, which resulted a reduction in the total cholesterol:HDL and LDL:HDL ratios. However, their participants were also in a weight-loss intervention programme. Therefore, there is a possibility that the improvement in LDL was attributed to the weight-loss intervention, which had a positive influence on cardiometabolic risk factors (Milsom et al., 2014; Spring et al., 2014; Vetter et al., 2013). Asemi et al. (2014) on the other hand, found that VLDL and TG were improved after eight weeks of intervention with 1000 mg of calcium daily combined with 50,000 IU of vitamin D weekly given to overweight women with vitamin D deficiency. However, no improvement was observed in other lipid profiles, including LDL. Low calcium intake increases intracellular calcium in hepatocytes, which stimulates microsomal triacylglycerol transfer protein (MTP), which is involved in the

formation of VLDL and TG (Van der Meer et al., 1990; Vaskonen, 2003). Therefore, the positive results regarding VLDL and TG found by Asemi et al. (2014) could be attributed to high calcium dietary intake (1110.9 ± 167.1 mg/d)in addition to vitamin D and calcium co-supplementation (50,000 IU of vitamin D per week plus 1000 mg of calcium per day) in the intervention group.

5.3.4.3 High density lipoprotein (HDL)

Although clinical trials have produced inconsistent results in lipid profiles, almost all trials, including ours, have failed to demonstrate an improvement in HDL with vitamin D supplementation (Asemi et al., 2014; Breslavsky et al., 2013; Gepner et al., 2012; Jorde et al., 2010; K. C. Maki et al., 2011; Patel et al., 2010; Sollid et al., 2014; Talaei et al., 2013; Witham, Dove, et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009).

Theoretically, an improvement in HDL was attributed to vitamin D inducing serum PTH suppression, thus reducing lipolysis, as well as an effect of vitamin D on insulin secretion and insulin sensitivity, which may indirectly influence lipid metabolism (Jorde & Grimnes, 2011). Only one study, by Major et al. (2007) demonstrated a reduction in the total cholesterol:HDL and LDL:HDL ratios. Nonetheless, the HDL concentration alone was not improved significantly in this particular trial. However, these findings should be interpreted with caution because the aim of the study was to evaluate the effect of calcium with vitamin D supplementation on an energy-restricted weight loss intervention. As explained in section 5.3.4.2, there is a possibility that the energy restriction from the weight-loss intervention may have influenced the reduction in the total cholesterol:HDL and LDL:HDL ratios.

Nevertheless, similar to our study, many clinical studies have found that vitamin D did not appear to significantly improve HDL (Jorde et al., 2010; Sollid et al., 2014; Wood et al., 2012; A. Zittermann et al., 2009). A non-significant reduction in HDL (-0.14 mg/dl) was reported in a meta-analysis (H. Wang et al., 2012). One possible reason is that all of these studies used circulating serum 25(OH)D to assess the link between HDL and vitamin D. Low HDL was associated with low circulating serum 1,25(OH)D, but no association was found between circulating serum 25(OH)D and HDL (Karhapaa et al., 2010). Perhaps serum 1,25(OH)D might be more appropriate for assessing links between vitamin D and HDL compared to serum 25(OH)D. It is known that 25(OH)D is synthesized in the liver, whereas 1,25(OH)D is synthesized in the kidney. Therefore, there is a possibility that HDL had an "extra-hepatic" link with 1,25(OH)D, whereas the association between 25(OH)D and cholesterol could be related to its synthesis in the liver because both cholesterol and 25(OH)D share a common 7-dehydrocholesterol pathway in the liver. However, 1,25 vitamin D has a short half-life and is thus difficult to measure.

5.3.4.4 Triglycerides (TG)

In our study, vitamin D appeared to have no significant effect on TG. Nevertheless, TG levels were found to be within the normal range at baseline and after 12 months of follow-up.

As discussed in greater detail in section 2.2.8.3, the known mechanisms of the effects of vitamin D on TG may involve a role of vitamin D in calcium regulation. Vitamin D increases intestinal calcium absorption; as a result, more calcium would be absorbed in the intestine following the consumption of vitamin D supplements. For that reason, hepatic TG formation or secretion would be decreased via an effect on hepatocellular calcium. Another mechanism would be related to high calcium intake and vitamin D supplementation, which

might reduce the stimulation of MTP in the liver, which has been implicated in the formation of TG. Because these two mechanisms are calcium dependent, there is a possibility that studies providing vitamin D and calcium co-supplementation would observe a significant improvement in TG (Asemi et al., 2014; Major et al., 2007).

On the other hand, Zitterman et al. (2009) found a significant 13% reduction in serum TG in the participants in the intervention group, who received 3332 IU of vitamin D without oral calcium supplementation daily. The TG reduction in this study might have been attributed to its weight-loss intervention programme along with the vitamin D supplementation. The weight-loss programme itself may have influenced the lipid profile results, as discussed in section 5.3.4.2.

Another possible mechanism by which vitamin D could improve TG is via increased peripheral removal due to serum PTH suppression with the improvement in serum 25(OH)D. However, this phenomenon did not occur in our study; the TG level did not improve, although PTH was significantly suppressed in the intervention group. Consistent with our findings, a few other clinical trials also observed similar negative findings regarding the effect of vitamin D in relation to TG (Breslavsky et al., 2013; Gepner et al., 2012; Jorde et al., 2010; K. C. Maki et al., 2011; Nagpal et al., 2009; Patel et al., 2010; Sollid et al., 2014; Talaei et al., 2013; Wood et al., 2012).

A systematic review by Jorde and Grimnes (2011) concluded that most clinical trials examining vitamin D and lipids have produced negative results because the studies were not specifically designed to evaluate the relationship between vitamin D and lipids. In addition, most of the study participants had normal baseline lipid profiles. As a

consequence, those studies did not have sufficient power to detect the effect of vitamin D on lipid profiles, thus resulting in negative results.

5.3.4.5 Blood pressure (BP)

As discussed in section 2.2.8.4, the ability of vitamin D to suppress renin-angiotensin-system activity and its functions in improving the flow-mediated dilatation of the brachial artery may explain how vitamin D could protect against hypertension. In addition, vitamin D is known to suppress PTH and pro-inflammatory cytokine production, thus indirectly protecting against cardiovascular diseases. However, the inconsistent results of previous trial studies prevent the elucidation of these biological mechanisms.

Our study demonstrated no significant improvement in either SBP or DBP with vitamin D supplementation for 12 months. However, two studies have observed a significant improvement in SBP, whereas no improvement was found in DBP (Pfeifer et al., 2001; Witham, Dove, et al., 2010). Participants who received co-supplementation of 800 IU/day of vitamin D and 1200 mg of calcium exhibited a 9.3% reduction in SBP compared to participants who received calcium alone (Pfeifer et al., 2001).

SBP has been improved with high doses of vitamin D alone (Witham, Dove, et al., 2010). That study did not combine calcium with vitamin D. A possible reason for this positive result could be that SBP was abnormal at baseline (SBP > 140 mmHg). As reported in a meta-analysis, no reductions were observed in studies with participants who were normotensive at baseline (Witham et al., 2009). The same meta-analysis also reported that in studies with elevated BP at baseline, a significant change was observed in DBP (mean difference: -3.1; 95% CI: -5.5 to -0.6 mmHg). On the other hand, a small but insignificant reduction was observed in SBP (mean difference: -3.6; 95% CI: -8.0 to 0.7

mmHg). This finding might explain why we did not observe any reduction in BP; the baseline BP in our study was within the normal range. Similarly, no improvement in BP was observed in other studies with participants who were normotensive at baseline (Gepner et al., 2012; Jorde et al., 2010; Major et al., 2007; Kevin C. Maki et al., 2009; Nagpal et al., 2009; Sollid et al., 2014; Strobel et al., 2014; von Hurst et al., 2010; Wood et al., 2012; Yiu et al., 2013; A. Zittermann et al., 2009).

Although laboratory studies have shown an effect of vitamin D on suppressing renin-angiotensin system activity (Yuan et al., 2007), previous trials were still unable to explain the biological mechanism of the effect of vitamin D on BP because most of them observed a non-significant reduction in BP. Therefore, the effect of vitamin D in relation to BP is questionable. Further research may be required to validate the effect of vitamin D on BP in hypertensive patients, particularly in participants with abnormal BP at baseline. Because our study was conducted in a community setting, only 8.9% of the participants were hypertensive, and most of them were on hypertension treatment and had normal BP at baseline. Therefore, it was difficult to obtain a significant improvement in BP after vitamin D supplementation because BP was already within normal values for the majority of our participants.

5.3.4.6 Metabolic syndrome

There was a small reduction in the proportion of metabolic syndrome in the intervention group, whereas there was a small increase in the placebo group. However, the difference between the groups was not significant ($p = 0.762$). Our results may be due a sample size that was not sufficiently large to detect an effect of vitamin D supplementation on metabolic syndrome. In addition, only a small proportion of our participants were at risk of metabolic syndrome as our participants were from a non-clinical setting. Based on the

National Health and Morbidity Survey IV (NHMS IV) conducted in 2011, only 14.5% of women aged more than 18 years were diabetic, whereas 31.6% were hypertensive (Institute of Public Health, 2011). Therefore, we had difficulty recruiting women with both vitamin D deficiency and metabolic syndrome in our setting.

One of our findings worth mentioning is the reduction in the proportion of metabolic syndrome in the intervention group compared to the placebo group, although it was not significant. Out of five metabolic syndrome risk factors, central obesity was the most common characteristic, followed by high BP (or on hypertension treatment) in both groups. There was also no improvement in the proportion of metabolic syndrome risk factors, except for low HDL level. Although it was not significant, our finding is consistent with a cohort study by Al-Daghri et al. (2012) which found that vitamin D status correction reduced metabolic syndrome from 25% to 13% by decreasing the proportions of low HDL and elevated BP.

In addition, Gagnon et al. (2012) suggested that IR mediated the association between metabolic syndrome risk and serum 25(OH)D in participants with BMI greater than 25 kg/m^2. Because there was no significant improvement in HOMA-IR in our study, there was no reduction in metabolic syndrome risk, although the majority of our participants in both the intervention and placebo groups were overweight (mean BMI: 27.23 ± 5.49 and 27.23 ± 5.09 kg/m^2, respectively).

Nevertheless, the studies by both Gagnon et al. (2012) and Al-Daghri et al. (2012) were not randomized controlled trials; therefore, a link between low serum 25(OH)D concentrations and future risk of developing metabolic syndrome could not be demonstrated because there was no comparison or control group. Further randomized

controlled trials designed to specifically detect the effect of vitamin D on the risk of developing metabolic syndrome need to be conducted to confirm these findings. There is still a lack of randomized controlled trial studies to establish causality between vitamin D and metabolic syndrome, although many observational studies have established an association between vitamin D and metabolic syndrome (Chacko et al., 2011; Moy & Bulgiba, 2011; Oosterwerff et al., 2011).

5.3.5 Effects of vitamin D supplementation on HRQOL

Vitamin D supplements did not improve the HRQOL of our participants. Although there were some improvements in the vitality and mental component scores, these improvements were statistically significant but clinically insignificant. The between group difference at 12 months was largely contributed by the higher score at baseline for both vitality score and MCS among the intervention group participants. However, the mental component score does have some clinical relevance because vitamin D deficiency has been associated with a range of neurological diseases, including depression, cognitive decline, Alzheimer's disease and Parkinson's disease (DeLuca, Kimball, Kolasinski, Ramagopalan, & Ebers, 2013; Eyles, Burne, & McGrath, 2013). Patients with vitamin D deficiency have also been reported to have low energy, fatigue and a feeling of always being tired. Vitamin D is a nuclear steroid hormone thought to be involved in brain health and neuromuscular functions. VDR in the cell's nucleus regulates the expression of target genes when bound to 1,25(OH)D. These receptors are expressed in areas of the brain important for behavioural regulation (Kalueff & Tuohimaa, 2007). Therefore, there is a possibility that the function of vitamin D as neurosteroid might have some effect on mental function and, consequently, health-related quality of life (HRQOL).

Although there was no improvement in the physical component of HRQOL with vitamin D supplementation in our study, vitamin D is thought to have some role in physical health through two mechanisms. Firstly, in addition to its function as a neurosteroid, VDR is also found in skeletal muscle fibres. The activation of VDR in skeletal muscle fibres is proposed to cause muscle growth and proliferation. Secondly, calcium and vitamin D supplementation also improves the function of the lower extremities and the size and number of muscle fibres (H. A. Bischoff-Ferrari et al., 2004; Boland, 1986; Simpson, Thomas, & Arnold, 1985). Therefore, theoretically, vitamin D supplementation should improve both physical and mental health and consequently improve the HRQOL in patients with vitamin D deficiency.

Many observational studies have reported an association between vitamin D and HRQOL (Anand et al., 2011; Sibel Basaran et al., 2007; Chao et al., 2014; Ecemis & Atmaca, 2013; W. Huang et al., 2013; Motsinger et al., 2012; Rafiq et al., 2014). However, very limited clinical trials have studied the effect of vitamin D on HRQOL. The available clinical trials studying the effect of vitamin D on HRQOL were mostly restricted to disease-specific populations, such as the osteoporotic elderly (Kenny, Biskup, Robbins, Marcella, & Burleson, 2003; Porthouse et al., 2005) or elderly with heart failure (Witham, Crighton, et al., 2010b). In addition, many trials have studied the effects of vitamin D on specific mental or physical illnesses, such as depression (Jorde et al., 2008; Kjaergaard et al., 2012), instead of overall HRQOL outcome.

An observation study by Ecemis and Atmaca (2013) found that the physical component, mental component, physical functioning and vitality scores were impaired in vitamin D-deficient and -insufficient healthy premenopausal women. Similar findings were reported in a case series study by Huang et al. (2013), in which vitamin D supplementation was found to improve HRQOL components, such as bodily pain, general health, vitality and

social functioning, in patients with chronic pain. Observational studies have reported that insufficient vitamin D status was a significant determinant of HRQOL in osteoporotic elderly women (Sibel Basaran et al., 2007; Motsinger et al., 2012; Ohta, Uemura, et al., 2014; Rafiq et al., 2014). A study by Chao et al. (2014) observed that an increase in serum 25(OH)D to 100 nmol/l was associated with a 29% improvement in HRQOL. A cross-sectional study of dialysis patients noted with every 25% reduction in serum 25(OH)D concentration was associated with a one-point reduction in the SF12 MCS score, but no association was found between PCS and vitamin D status (Anand et al., 2011).

Nevertheless, clinical trials have not found any beneficial effects of vitamin D in relation to HRQOL (Kenny et al., 2003; Porthouse et al., 2005; Witham, Crighton, et al., 2010b). However, the study populations in all three trials were elderly, and different sets of HRQOL questionnaires were used to measure HRQOL outcomes. In addition, different vitamin D supplementation formulations might influence outcomes. Kenny et al. (2003) prescribed 1000 IU of vitamin D with 500 mg of calcium daily to their participants for six months, and the SF8 questionnaire were used to measure the HRQOL outcomes. A daily dose of 800 IU of vitamin D with 1000 mg of calcium was used as the intervention in a trial by Porthouse et al. (2005) involving the SF12 questionnaire, whereas Witham et al. (2010b) used the Minnesota Living with Heart Failure Questionnaire to measure the effect of a single dose of vitamin D (100,000 IU) on HRQOL. Because all of these clinical trials used different sets of questionnaires for assessment, it is difficult to compare the findings. Although the SF12 and SF8 questionnaires are shorter versions of the SF36 questionnaire, the SF36 questionnaire produced better measurement precision compared to SF12 and SF8 (Optum.com, 2014). The Minnesota Living with Heart Failure Questionnaire was specifically designed to measure QOL in participants with heart failure, whereas SF36 is used in community settings.

Although clinical trials have been unable to observe any effect of vitamin D in relation to mental health, many systematic reviews and meta-analyses have reported that vitamin D deficiency was a plausible biological cause of neuropsychiatric disorders such as depression (Ju et al., 2013; May et al., 2010; Penckofer et al., 2010; Shaffer et al., 2014). However, there is a possibility that such results associated with depression were observed due to residual confounding. It is known that people with depression or emotional dysfunction are prone to reduced outdoor activity, which in turn could cause vitamin D insufficiency.

At baseline, our study showed slightly higher scores on the bodily pain component compared to the general population (Azman et al., 2003). Because all of our participants were vitamin D deficient at baseline, higher scores on bodily pain were unexpected, as muscle and bone pain is a common symptom of vitamin D deficiency. Nevertheless, we did not find any significant improvements in bodily pain or any other physical component domains after 12 months of treatment, which is in contrast to some clinical trials (W. Huang et al., 2013; Sakalli et al., 2012; Schreuder et al., 2012). A possible explanation for this discrepancy could be the non-specific definition of pain used in the SF-36 questionnaire that we administered. For example, Huang et al. (2013) used the VR-36 (VeteranRand 36 items) questionnaire and a VAS (visual analogue scale) to evaluate bodily pain and found that vitamin D supplementation was effective at alleviating pain and improving other components of HRQOL. Similarly, Sakalli et al. (2012) used a VAS and the SF-36 questionnaire to evaluate levels of pain, and they reported that a single mega-dose of vitamin D was effective at increasing QOL, particularly physical functioning, physical role functioning, bodily pain, general health, and social functioning as well as decreasing non-specific musculoskeletal pain. Schreuder et al. (2012) used only the VAS score to measure the intensity of non-specific musculoskeletal pain before and after an

intervention (a single dose of 150,000 IU of vitamin D versus placebo). They did not measure HRQOL as one of the study outcomes.

On the contrary, a study that used a disease-specific tool, such as the Minnesota Living with Heart Failure questionnaire, did not find any improvement in physical function or HRQOL in older patients with heart failure (Witham, Crighton, et al., 2010a). In that study, the questionnaire used was unable to measure pain precisely, and it was not combined with a VAS score to measure the intensity of pain.

5.3.6 Lifestyles practices over time

5.3.6.1 Dietary intake

The mean vitamin D intake of our participants over 12 months of follow-up was below the Malaysian RNI (RNI for vitamin D: 200 IU per day or 5 μg/day) in both treatment groups. There was no difference within or between the groups. In addition to UVB light from the sun, food sources are a natural source of vitamin D. The consumption of fatty fish, such as salmon, mackerel and cod liver oil, is a good alternative source of vitamin D, as are vegetables, such as mushrooms.

In addition to natural sources, many countries list margarine as a food item with mandatory vitamin D fortification (e.g., Malaysia, Singapore, and Canada), whereas some countries list foods, such as orange juice, milk and cheese products, as food items with voluntary vitamin D fortification (e.g., the United States and Philippines). However, none of these vitamin D-fortified foods or foods that are naturally rich in vitamin D are commonly consumed by Malaysians. Therefore, it is not possible for Malaysians to obtain sufficient vitamin D from foods, whether naturally or via vitamin D fortification. For that

reason, it is not surprising that the dietary vitamin D intake of our participants was lower than the Malaysian RNI.

Our findings are similar to a few other studies conducted in Malaysia (Istiany et al., 2012; Nurbazlin et al., 2013; Zalilah, Khor, Mirnalini, Norimah, & Ang, 2006). Nurbazlin et al. (2013) found that women living in urban areas had a lower vitamin D intake [median: 4.61 (2.66 to 7.42) µg per day] with a median (Q1 to Q3) serum 25(OH)D concentration of 31.9 (26.1 to 45.5). However, there was no correlation between serum 25(OH)D concentration and vitamin D intake (Spearman's rho = 0.084, p = 0,095). In another trial in postmenopausal Malay women, the mean vitamin D intake and vitamin D status of all of the groups at baseline was 2.6 ± 1.8 µg/day; 49.8 ± 22.7 nmol/l (control group); 2.9 ± 1.7 µg/day; 57.1 ± 20.6 nmol/l (nutrition education group); 2.8 ± 1.9 µg/day; 50.1 ± 25.5 nmol/l (sun exposure group) and 3.4 ± 2.5 µg/day; 53.2 ± 23.7 nmol/l (nutrition education with sun exposure group) (Istiany et al., 2012). That study found that after four months of intervention, the vitamin D intake and serum 25(OH)D concentration of the nutrition education and sunlight exposure with nutrition education groups were significantly increased compared with baseline. This outcome indicates that vitamin D nutritional status can be improved by increasing the vitamin D intake through diet.

Another possible reason for low vitamin D intake could be underestimation by our participants when they reported their food intake in the diet diary. Although the participants received a brief session by a trained dietician on how to complete the diary, they might still have wrongly estimated serving sizes, thus causing inaccurate results in the dietary intake. In addition, the lack of a vitamin D database for local Malaysian foods could be a possible reason for the low vitamin D dietary intake in our study. We used the United States Department of Agriculture (USDA) Standard Reference Database to obtain the vitamin D

contents of raw foods. Therefore, there is a possibility that the vitamin D intake in our study could be under or over-estimated because the vitamin D contents of foods from the US and Malaysia may be different.

Regarding energy intake, our participants' mean values at baseline and after 12 months of follow-up were also lower than the RNI for female Malaysians. Nevertheless, our results were slightly higher than the findings of the Malaysian Adult Nutrition Survey (MANS), which reported a mean total energy intake of urban females of 1437 ± 15 kcal (Mirnalini et al., 2008). The major contributor to the total calories in our participants' diets was carbohydrate sources (50% to 60), followed by fat and protein (30% to 35 % and 15% to 16%, respectively). Similar findings were reported by the MANS study, which reported that carbohydrates contributed to 59% of the total energy intake. Similar to the MANS study, the calcium intake of our participants was lower than the Malaysian RNI (> 800 mg/day) at baseline and after 12 months of follow-up. There was no significant change in their dietary intake over time. Therefore, we assume that there is no diet modification over time that can affect both serum 25(OH)D concentrations and cardiometabolic risk factors.

5.3.6.2 Physical activity

There was no significant improvement in physical activity over time in our study. The majority of the participants in our study had a sedentary lifestyle. Most of them worked in the university administration office as clerks or administrators, desk jobs that did not require any vigorous physical activity. This finding was reflected in the sitting scores: our participants spent more than 300 minutes per day sitting. Because all of our participants were employed, it was expected that all of their main routine physical activities were in the work-related physical activity domain.

In addition to work-related physical activity, our findings suggest that physical activities related to domestic chores, followed by transportation, were more important sources of energy expenditure than leisure-time physical activity. More than 50% of our participants were in the moderate physical activity category. On most days, the majority of our participants performed only 30 minutes of at least moderate-intensity physical activity. Only fewer than 30% of our participants were in the high physical activity category. A similar physical activity pattern was observed in the findings from the Malaysian Adult Nutrition Survey (MANS), which reported that Malaysian women spent most of their time in the sitting position (mean 559.3, 95% CI: 552.9 to 565.7 minutes per day); sports activities, such as running and cycling, were their least common physical activities (mean: 4.3, 95% CI: 3.6 to 5.0 minutes per day) (Poh et al., 2010).

Possible reasons for these results include long working hours and demanding domestic chores, which limited the participants' time for leisure physical activity. Furthermore, our participants exhibited higher energy expenditures in the household domain than in leisure-time physical activity because women have traditionally engaged in household chores. In addition, low physical activity levels among people living in developing countries are largely due to work-related issues, such as long work commutes, more hours sitting at desk jobs and the use of public transportation (Pires et al., 2012).

Low physical activity, especially sports and recreational or leisure-time physical activity, is known to increase the risk of cardiovascular risk factors. Many studies have reported favourable results related to leisure-time physical activity and cardiometabolic risk factors (A. H. Y. Chu & Moy, 2014; Pires et al., 2012). Therefore, our participants were at risk of developing cardiometabolic diseases. However, there were no changes in physical activity over time in either group in this study that could lead to changes in cardiometabolic risk factors.

Some studies have suggested that physical activity may be associated with vitamin D status (Kluczynski et al., 2011; A. Zittermann et al., 2000). However, low physical activity may be a residual confounding factor for vitamin D deficiency. Leisure-time physical activities are commonly associated with outdoor sports, such as jogging or swimming, which might expose people to more sunlight, thus improving their vitamin D status. Therefore, people with low levels of leisure-time physical activity are vitamin D deficient not because physical activity is associated with vitamin D but because they tend to spend more time at home or indoors. De Rui et al. (2014) found that outdoor physical activities, such as cycling and gardening, were associated with a lower risk of a serum vitamin D level less than 50 nmol/l, whereas no association was found between indoor physical activity and vitamin D deficiency. For that reason, there is a possibility that vitamin D deficiency was not directly associated with physical activity but was instead due to physical activity exposing people to more or less sunlight, consequently affecting their vitamin D status.

5.3.6.3 Sunlight exposure

Malaysia is a tropical country that is blessed with abundant sunshine throughout the year. Sunlight is the primary source of vitamin D through 7-dehydrocholesterol activation from skin exposure to UVB radiation (Holick, 2011). Almost 95% of the body's vitamin D requirement comes from sunlight. Therefore, any factors that prevent UVB light absorption could influence vitamin D synthesis, thus causing vitamin D deficiency. Factors that may influence vitamin D synthesis from sunlight could be (1) latitude, season and time of day; (2) use of sunscreen or sunblock; (3) clothing style; and (4) melanin or skin pigmentation.

Many studies have reported that sunlight exposure at least two to three times per week for 10 to 15 minutes each time (20 to 45 minutes per week) resulted in sufficient vitamin D

synthesis by the skin (Dowdy et al., 2010; Holick, 2011). In the present study, participants in both groups were exposed to sunlight for more than 45 minutes per week at baseline, but they still had insufficient vitamin D concentrations. Similar findings were reported by Nurbazlin et al. (2013), who found that despite sun exposure of 2.92 hours / week, urban women had a low serum 25(OH)D concentration. These findings could be due to participants overestimating their sunlight exposure. A similar problem may have occurred in our study. McCarty (2008) concluded that sunlight exposure assessment through a questionnaire was a poor proxy for vitamin D status, which could be due to the imprecision of ultraviolet estimates obtained from questionnaires, which can be susceptible to recall bias. To date, no standard, validated sunlight questionnaires are available to quantify sunlight exposure. Another approach to assess the erythemally effective solar ultraviolet dose received by anatomical sites (personal exposure) is use of a polysulphone dosimeter. This method is well known, but it may not be feasible for community trials due to budget constraints.

Lifestyle practices that prevent UVB light absorption into the skin would also affect the vitamin D production in the body. Usage of sunscreen prevents the formation of pre-vitamin D. Similarly, wearing clothing that covers almost 95% of the body surface also prevents the maximum absorption of UVB into the skin (Holick, 2011). In addition, the amount or thickness of sunscreen applied, the SPF level of the sunscreen (Faurschou et al., 2012) and texture and colour of clothing (Matsuoka et al., 1992) may affect the penetration of UVB light into the skin; these factors were not addressed in further detail in our study. Therefore, it is not surprising that vitamin D deficiency is the most common in cultures that require almost the entire body to be covered with clothing, such as Muslim cultures, especially in females (Ahmed, Al-Murrani, Kuri, & Rees, 2013; Al-Elq, 2012; Al Attia &

Ibrahim, 2012; Faghih, Abdolahzadeh, Mohammadi, & Hasanzadeh, 2014; Golbahar et al., 2014; Moy, 2011). In the present study, most of the participants were more covered up due to religious practices because the majority of our participants were Muslim. However, the percentage of participants who applied sunscreen when exposed to the sun was less than 50% for both groups.

5.4 Strength and limitations of study

5.4.1 Limitations of the study

Although all of our participants were vitamin D deficient, only a small proportion were at risk of CVD because our study was conducted in a non-clinical setting. From the National Health and Morbidity Survey IV (NHMS IV) conducted in 2011, only 14.5% of women aged more than 18 years were diabetic, and 31.6% were hypertensive (Ministry of Health, 2011). Therefore, we had difficulty recruiting women with both vitamin D deficiency and CVD risk. Thus, our study may not have sufficient power to detect the effects of vitamin D supplementation on cardiometabolic risks. Sensitivity analysis on high risk participants (participants with metabolic syndrome at baseline) was carried out and similar results with the main analysis was obtained (Appendix L)

We were also unable to examine the incidence of cardiovascular events due to the short duration of our trial. A larger sample size of a population at high risk of CVD and a longer follow-up may be needed to establish whether the steady normalization of vitamin D levels will translate into a decrease in cardiovascular event incidence.

An article by Heaney (2003) indicated that vitamin D insufficiency may produce more than one disease by more than one mechanism. Several years may also be required for the consequent morbidity to be sufficiently evident as ‘‘disease’’. Similarly, stronger evidence

is needed to demonstrate that a higher intake of vitamin D than usual is required for disease prevention. For example, researchers took years to establish the effects of vitamin D in relation to osteoporosis, and osteoporosis prevention requires almost 4-fold the intake of vitamin D needed to prevent rickets. At present, there is no recommendation on vitamin D intake for cardiometabolic prevention due to a lack of evidence. It may take a longer time and more clinical trials to establish this knowledge.

The intervention in our study was 50,000 IU per week for eight weeks and 50,000 IU per month for 10 months based on the recommendation by Holick (2009). This formulation was chosen for its advantage on compliance compared to daily doses. However, we were unable to ensure that the vitamin D levels remained constantly above the sufficient level (> 75 nmol/l) throughout the study because we did not perform frequent testing.

Lifestyle practices, such as sunlight exposure history, physical activity and diet diary, were all self-reported. There is a possibility of a bias known as social desirability bias in which participants may provide answers that are expected by the investigators. However, the findings from the questionnaires were comparable between the groups before and after treatment. Therefore, there was no significant alteration in lifestyle practices over time that could affect the outcome measures in the present study.

In addition, the use of the SF36v2 questionnaire alone without combination with a VAS questionnaire may have failed to detect the effect of vitamin D on HRQOL, particularly bodily pain,as vitamin D deficiency is known to cause musculoskeletal pain and correcting the vitamin D status should decrease such pain.

In this study, the vitamin D contents of raw foods were obtained from the United States Department of Agriculture (USDA) Standard Reference Database and food product labels

because the Nutrient Composition of Malaysian Foods database does not provide vitamin D compositions for Malaysian foods. This aspect of the study may have caused an inaccurate estimation of vitamin D intake. Common statistical techniques for dietary analysis such as residual method of energy adjustment were not conducted to handle the inaccurate estimation of food consumed.

Although all of the appropriate end points for cardiometabolic risks were considered in this study, inflammatory markers considered predictors of cardiometabolic risks, such as apolipoprotein B (ApoB) and C-reactive protein (CRP), were not measured. ApoB may be a better predictor of CVD risk than LDL cholesterol, particularly the on-treatment LDL cholesterol level (Brunzell et al., 2008). CRP is a marker of inflammation and is directly involved in atherogenesis. Elevated CRP levels measured by highly sensitive assays (hs-CRP) are associated with increased cardiovascular risk (Pearson et al., 2003; Shishehbor, Bhatt, & Topol, 2003; Xu & Whitmer, 2006).

In addition, neither computerized tomography nor ultrasound scanning was performed in this study to observe the effects of vitamin D supplementation on carotid calcification or smooth muscle cell proliferation as cardiovascular risk factors. Carotid intima-medial thickness ultrasound has been used as a reliable surrogate end point in many therapeutic interventions, such as lipid-lowering drugs (Y. Huang, Li, Dong, Li, & Wu, 2013) and antihypertensive agents (Cuspidi, Negri, Giudici, Capra, & Sala, 2009; J. G. Wang et al., 2006). However, we did not use the above markers due to budget constraints, lack of availability and lack of standardization of measurement protocols for carotid intima-media. However, the outcome measures that were used in this study were known to be sufficient and feasible for trials conducted in a community setting.

5.4.2 Strengths of the study

Nevertheless, our study has several strengths that are worthy of mention. One strength is the high quality of randomization, which included concealment of allocation, double-blinding among the participants and researchers and a placebo-controlled design. This randomization reduced bias in terms of selection and information bias, as the self-reported HRQOL was subjective.

Larger but less frequent dosages of vitamin D may be advantageous from a compliance perspective compared to daily doses. In addition, our participants' retention rate was good; only 11% dropped out of the study. The socio-demographic comparison revealed no significant differences between the dropouts and the complete case participants.

There was also no problem with seasonal confounders as Malaysia is blessed with sufficient sunshine throughout the year necessary for cutaneous synthesis of vitamin D. Both groups had comparable levels of outdoor physical activity, sunlight exposure activity and diet at baseline, and there were no changes pre- or post-intervention.

To our knowledge, this study is the first randomized controlled trial to examine the effects of vitamin D on cardiometabolic risk factors and HRQOL in Malaysia. Many studies in Malaysia have demonstrated that despite its location at a latitude 03° 9'north, with UVB radiation of sufficient wavelength necessary for the cutaneous synthesis of vitamin D almost throughout the year, the vitamin D deficiency prevalence in Malaysia is still high (Istiany et al., 2012; Khor et al., 2011; Moy, 2011; Moy & Bulgiba, 2011; Nurbazlin et al., 2013). However, no studies have assessed the effects of vitamin D supplementation on cardiometabolic risk factors and HRQOL in vitamin D-deficient subjects in Malaysia. Therefore, this study can serve as a basis for further studies as this is the first study to assess the link between vitamin D and cardiometabolic risk factors and HRQOL in Malaysia.

CHAPTER 6 : CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In summary, all of the socio-demographic characteristics, including medical history and lifestyle practices, as well as the anthropometric, clinical and biochemical measurements were comparable between the two groups. The majority of the participants were in their early forties, were Malays and had at least a secondary education. Although 25% of the participants had co-morbidities, such as hypertension, diabetes mellitus and high cholesterol, their mean BMI was in the overweight range; only 1/4 of participants were in the normal weight range. Their mean serum 25(OH)D was approximately 30 nmol/l, with a serum PTH level in the range of 4.6 to 4.7 pmol/l. The mean values of other clinical and biochemical measurements were in the normal range, except for LDL, which was greater than 3.00 mmol/l with more than 80% of the participants having abnormal values. The mean scores on all of the HRQOL components ranged from 60 to 70. Physical functioning, physical role functioning and social functioning were found to be lower compared to the general population.

This study found that 93.2% of urban premenopausal women working in a public university were vitamin D deficient during the screening phase. However, only 55.8% of them consented to participate in this study. Among the participants in this study, 89% completed the follow-up. The rate of compliance with the intervention was good as the participants were monitored closely throughout the trial.

This trial showed that a larger dosage but a lower frequency was effective at improving serum vitamin D levels and had an advantage in terms of participants' compliance compared to a daily dosage. The mean serum 25(OH)D in the intervention group increased

drastically even after only six months of treatment (mean difference: 50.85, 95% CI: 45.51 to 56.18 nmol/l). A total of 94% of the intervention group achieved a serum 25(OH)D level greater than 50 nmol/l at the end of the trial.

Over the intervention period, there were no changes in lifestyle behaviours, such as sunlight exposure or dietary intake, in either group; thus, we can conclude that the increase in serum 25(OH)D concentration in the intervention group was mainly due to the vitamin D supplementation. However, improving vitamin D status through supplementation for one year had no meaningful effects on lipid profiles, HOMA-IR, blood pressure or HRQOL.

6.2 Recommendations and public health significance

6.2.1 Recommendations for clinical / public health actions

The findings of this study show that vitamin D supplementation can be the most reasonable solution to improve the vitamin D status in the community. We also demonstrated that large and less-frequent doses of vitamin D supplementation provided greater advantages from a compliance perspective compared to daily doses and are thus advisable, especially in high-risk populations such as the elderly. To improve the vitamin D status in the obese, higher doses of vitamin D supplementation might be advisable because such patients might need a two- to three-fold higher dosage to maintain a sufficient serum 25(OH)D concentration. Nevertheless, we need to learn more about the long-term safety and efficacy of high dosages.

To receive more beneficial effects on cardiometabolic risk prevention, combinations of vitamin D and calcium should be used, rather than giving vitamin D alone. This recommendation is to ensure that dietary calcium and vitamin D are adequate, thus resulting in improvements in cardiometabolic risk factors.

Recommendations for vitamin D supplements may be questioned because sunlight exposure is the least expensive and easiest way to obtain vitamin D. However, it is difficult to recommend sunlight exposure to improve vitamin D levels in our setting due to cultural and religious reasons as well as the risk of skin cancer due to excessive UV radiation. An RCT by Wicherts et al. (2011) found that vitamin D supplementation was more effective than sunlight exposure advice for vitamin D deficiency treatment. In addition, skin pigmentation may be another reason why sunlight exposure may not work for Malaysians. Therefore, supplementation is the best solution for Malaysians as the majority of Malaysian skin colours are categorized as skin type 4 (brown) to type 5 (dark brown) by the Fitzpatrick Classification Scale, which possess a high ability to produce melanin, which acts as sunlight protection, thus reducing vitamin D synthesis in the skin.

In addition to natural food sources, one of the best ways of preventing vitamin D deficiency via dietary intake is by food fortification. However, all of the available vitamin D-fortified foods in the market or foods that are naturally rich in vitamin D are not commonly consumed by Malaysians. A recent review found that vitamin D fortification using vegetable oil can be an efficacious way to increase the daily intake of vitamin D in Southeast Asian countries (Yang et al., 2013). Therefore, it is recommended that mandatory vitamin D fortification of vegetable oil should be implemented.

Vitamin D deficiency is becoming more prevalent not only in populations living in temperate latitudes but also in tropical countries. Vitamin D deficiency has now received increased attention not only due to its implications for bone health but also for extraskeletal health, such as cardiometabolic diseases and their risk factors and cancer. Physicians should be alert to the signs and symptoms of vitamin D deficiency, especially in high-risk populations, such as Muslim women and women who work indoors. These populations should have routine screening, and early prevention should be started for them not only to

preserve bone and muscle health but also to possibly help preserve overall health and well-being.

Policy makers should perform surveillance of vitamin D deficiency, especially in high-risk populations. By doing this, we would be able to monitor the vitamin D status in the community, and early prevention activities could be started to prevent cardiometabolic diseases and to improve HRQOL in future.

6.2.2 Recommendations for future research

More interventional studies are required to confirm the effects of vitamin D on cardiometabolic risk factors, particularly in pre-diabetic or pre-hypertensive patients, and to evaluate the potential role of vitamin D in primary prevention of cardiometabolic risks. The trials should be performed over a longer duration, i.e., more than five years, as cardiometabolic risks take years to develop, depending on risk factors. A larger sample size of a high-risk population for CVD with longer follow-up and higher doses of vitamin D supplementation may be necessary to establish whether the steady normalization of vitamin D levels will translate to a decrease in cardiovascular event incidence. Regimens with different vitamin D dosages and frequencies (daily versus weekly or monthly) should be performed. Trials of co-supplementation with calcium are also needed. More frequent testing of vitamin D is also needed to observe when vitamin D reaches the highest level and when it starts to plateau.

As mentioned previously, few clinical trials have studied the effects of vitamin D in relation to HRQOL in a community setting. Because vitamin D deficiency can affect everyone regardless of their age or illness, more studies should focus on everyone, especially young adults, for the early prevention of vitamin D deficiency.

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LIST OF PUBLICATIONS

The following papers have been published or presented from this thesis:

Peer Reviewed Journal:

1. Mazliza Ramly, Moy Foong Ming, Suhaili Suboh, Alexander Tan Tong Boon, Rokiah Pendek. Study protocol: The Effect of Vitamin D Supplements on Cardiometabolic Risk Factors among Urban Premenopausal Women in a Tropical Country – A Randomized Controlled Trial. *BMC Public Health,*2013*;* 13:416

2. Mazliza Ramly, Moy Foong Ming, Suhaili Suboh, Karuthan Chinna, Rokiah Pendek. The Effect of Vitamin D Supplements on Cardiometabolic Risk Factors and Health-Related Quality of Life among Urban Premenopausal Women in a Tropical Country – A Randomized Controlled Trial. *Plos One,* 2014 Oct 28;9(10):e110476

Presentation at seminars or conferences:

1. Mazliza Ramly, Moy Foong Ming, Suhaili Suboh, Rokiah Pendek. A Randomized Controlled Trial of Vitamin D Supplements' effect on Cardiometabolic Risks and Quality of Life among Premenopausal Women: An Interim Analysis. Accepted for seminar presentation in the 7th Scientific Seminar: Update on Vitamin D in Human Nutrition, ILSI Southeast Asia Region Malaysia Country Committee, Kuala Lumpur, 12th November 2013.

2. Mazliza Ramly, Moy Foong Ming, Suhaili Suboh, Karuthan Chinna, Rokiah Pendek The Effect of Vitamin D Supplements on Cardiometabolic Risk Factors and Health-Related Quality of Life among Urban Premenopausal Women in a Tropical Country – A Randomized Controlled Trial. Accepted for presentation in the 46th Asia Pacific Consortium of Public Health Conference (APACPH), Kuala Lumpur, 16th – 19th October 2014.