FACTORS ASSOCIATED WITH DIFFERENCES IN VITAMIN D LEVELS BETWEEN THE URBAN AND RURAL POPULATION OF MALAYSIA

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Abstract

Background: UV-B sunlight exposure is a primary source of vitamin D. There have been reports of low vitamin D status amongst the Malaysian population despite it being a tropical country. This study was conducted to determine the vitamin D status in urban and rural women in Malaysia and factors predicting 25(OH)D levels.

Methods: Serum 25(OH)D was measured along with demographic and physical characteristics, intact parathyroid hormone (i-PTH), sun exposure and dietary vitamin D and calcium intake in urban and rural women aged above 45 years.

Results: Median (Q25-Q75) age of the subjects was 57 (53-61) years. Rural women had significantly higher median (Q25-Q75) levels of 25(OH)D compared to urban women [69.50 (58.95-79.14) vs 31.90 (26.05-45.50) nmol/L, p<0.001]. Majority of urban women (43.9%) had vitamin D deficiency (<30 nmol/L), while most of rural women (88.1%) were vitamin D sufficient (≥50 nmol/L). Although rural women had significantly lower fraction of body surface area (BSA) exposed to sunlight compared to urban women [0.12 (0.07-0.17) vs 0.21 (0.21-0.43), p<0.001], but they spent a lot of time in the sun compared to urban women [7.83 (3.67-14.71) vs 2.92 (1.17-4.92) hours, p<0.001], leading to significantly higher calculated sun index (hours of sun exposure per week×fraction of BSA) compared to urban women [0.89 (0.42-1.83) vs 0.72 (0.26-1.28), p=0.018]. Median (Q25-Q75) vitamin D intake of rural women was higher compared to urban women [5.23 (3.31-8.45) vs 4.61 (2.66-7.41) µg/day, p=0.050]. Rural women also had significantly higher median (Q25-Q75) intake of calcium compared to urban women [529.84 (328.17-736.09) vs 423.49 (324.28-561.67) mg/day, p=0.001] respectively. Only 7.5% of urban women and 19.1% of rural women met the

recommended nutrient intake (RNI) for vitamin D. A total of 8.9% of rural women met the RNI for calcium compared to 0.9% of urban women. Significantly positive correlation were found between waist circumference (rho=0.114, p=0.023), darkest (rho=0.137, p=0.006) and delta (rho=0.115, p=0.022) skin colour score, hours of sun exposure per week (rho=0.342, p<0.001), sun index (rho=0.180, p<0.001) and dietary calcium intake (rho=0.162, p=0.001) with serum 25(OH)D levels. Conversely, age (rho=-0.028, p<0.001), household income (rho=-0.224, p<0.001) and fraction of BSA exposed to sunlight (rho=-0.264, p<0.001) were negatively correlated with serum 25(OH)D levels. However, there were no significant correlation between BMI (p=0.248), body fat percentage (p=0.061), lightest skin colour score (p=0.212), serum i-PTH (p=0.342) and dietary vitamin D intake (p=0.095) with serum 25(OH)D levels. In the stepwise linear regression, rural dwelling, being Chinese and use of vitamin D containing supplements increased the serum 25(OH)D by 37.09, 9.72 and 6.11 nmol/L respectively. Moreover, 25(OH)D levels increased by 0.29 nmol/L for every unit increment in hours of sun exposure per week.

Conclusion: Rural women in Malaysia had significantly higher vitamin D levels compared to urban women. Higher sun exposure and better dietary intake of vitamin D and calcium may have contributed to the differences in vitamin D status. Rural dwelling, being Chinese, hours of sun exposure per week and use of vitamin D containing supplements were key factors influencing vitamin D status in Malaysian women.

Key Words: 25(OH)D, sun exposure, dietary vitamin D and calcium intake, rural and urban women, Malaysia

Abstrak

Latar Belakang: Pendedahan kepada cahaya UV-B adalah sumber utama vitamin D. Walaupun Malaysia adalah sebuah Negara Tropika, tetapi terdapat laporan berkenaan status vitamin D yang rendah di kalangan populasi di Malaysia. Kajian ini dijalankan bagi menentukan status vitamin D di kalangan wanita bandar dan luar bandar di Malaysia dan faktor-faktor yang meramalkan paras 25(OH)D.

Kaedah: Serum 25(OH)D diukur bersama-sama dengan ciri-ciri fizikal dan demografik, intact parathyroid hormone (i-PTH), pendedahan kepada cahaya matahari dan pengambilan makanan yang mengandungi vitamin D dan kalsium di kalangan wanita bandar dan luar bandar berumur 45 tahun dan ke atas.

Keputusan: Median (Q25-Q75) umur subjek adalah 57 (53-61) tahun. Wanita luar bandar mempunyai paras 25(OH)D yang signifikan tinggi berbanding wanita bandar [69.50 (58.95-79.14) vs 31.90 (26.05-45.50) nmol/L, $p<0.001$]. Majoriti wanita bandar (43.9%) adalah kekurangan vitamin D (<30 nmol/L), manakala kebanyakan wanita luar bandar (88.1%) mempunyai vitamin D yang mencukupi (≥50 nmol/L). Walaupun wanita luar bandar mempunyai kurang pecahan bahagian permukaan badan (BSA) yang terdedah kepada cahaya matahari berbanding wanita bandar [0.12 (0.07-0.17) vs 0.21 (0.21-0.43), $p<0.001$], namun mereka memperuntukkan banyak masa di bawah cahaya matahari berbanding wanita bandar [7.83 (3.67-14.71) vs 2.92 (1.17-4.92) jam, $p<0.001$], menghasilkan kiraan indeks cahaya matahari yang signifikan tinggi (jam pendedahan kepada cahaya matahari setiap minggu×pecahan BSA) berbanding wanita bandar [0.89 (0.42-1.83) vs 0.72 (0.26-1.28), $p=0.018$]. Median (Q25-Q75) pengambilan makanan yang mengandungi vitamin D bagi wanita luar bandar adalah

tinggi berbanding wanita bandar [5.23 (3.31-8.45) vs 4.61 (2.66-7.41) µg/day, p=0.050]. Wanita luar bandar juga mempunyai median (Q25-Q75) pengambilan makanan yang mengandungi kalsium yang signifikan tinggi berbanding wanita bandar [529.84 (328.17-736.09) vs 423.49 (324.28-561.67) mg/day, p=0.001] masing-masing. Hanya 7.5% daripada wanita bandar dan 19.1% daripada wanita luar bandar melepasi pengambilan nutrien yang disyorkan (RNI) bagi vitamin D. Sebanyak 8.9% daripada wanita luar bandar melepasi RNI bagi kalsium berbanding 0.9% daripada wanita bandar. Terdapat korelasi positif yang signifikan antara ukur lilit pinggang (rho=0.114, p=0.023), skor warna kulit paling gelap (rho=0.137, p=0.006) and delta warna kulit (rho=0.115, p=0.022), jam pendedahan kepada cahaya matahari setiap minggu (rho=0.342, p<0.001), indeks cahaya matahari (rho=0.180, p<0.001) dan pengambilan makanan mengandungi kalsium (rho=0.162, p=0.001) dengan paras serum 25(OH)D. Sebaliknya umur (rho=-0.028, p<0.001), pendapatan isi rumah (rho=-0.224, p<0.001) dan pecahan BSA yang terdedah kepada cahaya matahari (rho=-0.264, p<0.001) mempunyai korelasi negatif dengan paras serum 25(OH)D. Walaubagaimanapun, tiada korelasi yang signifikan antara BMI (p=0.248), peratusan lemak badan (p=0.061), skor warna kulit yang paling cerah (p=0.212), serum i-PTH (p=0.342) dan pengambilan makanan yang mengandungi vitamin D (p=0.095) dengan paras serum 25(OH)D. Melalui regresi linear berperingkat, kediaman luar bandar, merupakan kaum Cina dan penggunaan suplemen yang mengandungi vitamin D masing-masing meningkatkan serum 25(OH)D kepada 37.09, 9.72 dan 6.11 nmol/L. Tambahan lagi, paras 25(OH)D meningkat sebanyak 0.29 nmol/L bagi setiap unit pertambahan jam pendedahan kepada cahaya matahari setiap minggu.

Kesimpulan: Wanita luar bandar di Malaysia mempunyai paras vitamin D yang signifikan tinggi berbanding wanita bandar. Pendedahan kepada cahaya matahari yang

tinggi dan pengambilan makanan mengandungi vitamin D dan kalsium yang baik mungkin menyumbang kepada perbezaan status vitamin D. Kediaman luar bandar, merupakan kaum Cina, jam pendedahan kepada cahaya matahari setiap minggu dan penggunaan suplemen yang mengandungi vitamin D merupakan faktor utama yang mempengaruhi status vitamin D bagi wanita Malaysia.

Kata Kunci: 25(OH)D, pendedahan kepada cahaya matahari, pengambilan makanan yang mengandungi vitamin D dan kalsium, wanita bandar dan luar bandar, Malaysia

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Table of Contents

List of Figures

List of Tables

List of Symbols and Abbreviations

<	less than
>	greater than
≤	less than or equal to
≥	greater than or equal to
α	alpha
µg/day	microgram per day
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
5DDR	5-Day Diet Record 5DDR
7-DR	7-day dietary record
24-HDRs	24-hour dietary recalls
25(OH)D	25-hydroxyvitamin D
BIA	Bioelectrical Impedance Analysis
BMD	Bone Mineral Den sity
BMI	Body mass index
BSA	Body Surface Area
C	Carbon
CI	Confidence Interval
CKD	Chronic kidney disease
CLD	Chronic liver disease
cm	centimeter
CV	Coefficient of variation
ECLIA	Electrochemiluminescence immunoassay
FELDA	Federal Land Development Authority
FFQ	Food Frequency Questionnaire

g	gram
g/wk	gram/weak
H_a	Research Hypothesis
H_0	Null Hypothesis
HPLC	High-performance liquid chromatography
i-PTH	intact-parathyroid hormone
J	Joule
J/m^2	joule per square meter
kg	kilogram
kg/m^2	kilogram per square meter
IOF	International Osteoporosis Foundation
IOM	Institute of Medicine
IU	International Unit
L	liter
LC-MS	Liquid Chromatography-Mass Spectrometry
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
LSES	Lower socioeconomic strata
m	meter
m^2	square meter
MEC	Medical Ethics Committee
N	North
NCCFN	National Coordinating Committee on Food and Nutrition
ng/ml	nanogram per milliliter
NHS	Nurses' Health Study
nmol/L	nanomole per liter
OR	Odd ratio

PS	polysulphone
PTH	Parathyroid hormone
Q25-Q75	quartile 25-quartile 75
RDA	Recommended Daily Allowance
RIA	Radioimmunoassay
RNI	Recommended Nutrient Intakes
RPM	Revolutions per minute
S	South
SD	Standard deviation
SES	Socioeconomic status
TBF	Total body fat mass
UMMC	University of Malaya Medical Centre
US	United States
USDA	United States Department of Agriculture
USES	Upper socioeconomic strata
UV	Ultraviolet
UVB	Ultraviolet Beta
VIF	variance inflation factor
vs	versus
VSMC	Vascular smooth muscle cells

List of Appendices

CHAPTER I

1.0 INTRODUCTION

1.1 Background

Vitamin D was discovered as a vitamin in the early 1920s (Norman, 1998). However, since the characteristics and structure of its most bioactive derivative, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] is similar to other steroid hormones; as a result, it is recognized as a prohormone rather than a true vitamin (Thacher, et al. 2011). It acts as a hormone, it stimulates the uptake of calcium and phosphate from the intestines to promote their release from the bones and to reduce calcium loss from the kidneys. This, leads to increased levels of blood calcium and phosphate.

Vitamin D is important for bone health. Vitamin D deficiency can cause rickets in children and osteomalacia in adults. Deficiency in vitamin D is associated with increase risk of bone loss and osteoporotic fracture in older people (Schoenmakers, et al. 2008). Recent findings reported that low vitamin D levels is associated with the development of multiple sclerosis, type 1 diabetes, implications to the immune system (Burgaz, et al. 2007), cancer (e.g. colorectal cancer) (Feskanich, et al. 2004), high blood pressure (Forman, et al. 2007), cardiovascular disease (Wang, et al. 2008) and chronic liver disease (Gordon, et al. 2004). On the other hand, large amounts of vitamin D may cause hypercalcemia, nephrocalcinosis and kidney failure (Pedersen, 2008).

Vitamin D can be obtained from the endogenous production of vitamin D3 (cholecalciferol) formed by the activation of 7-dehydrocholesterol through the exposure

of the skin to ultraviolet B (UVB) radiation. It also can be derived from the exogenous sources, D3 and D2 (ergocalciferol) that are found in the diet, vitamin D–fortified foods and supplements (Burgaz, et al. 2007). Foods that contain vitamin D include fish (e.g. mackerel, salmon, sardines and tuna), liver, egg yolks, and dairy foods (e.g. milk, yogurt, cheese and butter).

The Recommended Nutrient Intake (RNI) of vitamin D and calcium for the Malaysian population is based on age. Malaysian women aged 30-50, 51-65 and >65 years need 5 µg/day (200 IU/day), 10 µg/day (400 IU/day), and 15 µg/day (600 IU/day) of vitamin D respectively. In contrast, Malaysian women aged 30-50 and above 51 years need 800 and 1000 mg/day of calcium respectively (Mohd Ismail Noor, et al. 2005). Lee (2011) found that only 12% of the older adults in the Klang Valley met the RNI requirement for vitamin D. The low percentage of vitamin D intake may be due to an inadequate consumption of milk, the practice of a vegetarian diet and very little or no supplement intake (Whiting, et al. 2005).

Vitamin D status can be determined by 25-hydroxyvitamin D [25(OH)D], the major circulating form of vitamin D (Holick, 2007). According to Schoenmakers, et al. (2008), 25(OH)D is usually used as an indicator of vitamin D status because it has a long half-life and is not under tight homeostatic regulation and therefore reflects vitamin D supply and usage over a period of time. Various cut-off points are use in previous research to determine vitamin D deficiency/insufficiency.

Table 1.1 : Classification of Vitamin D Status by 25(OH)D Levels

	25(OH)D				
References	Deficient	Insufficient	Sufficient	Suboptimal	Optimal
IOF[1]	<25 nmol/L (10 ng/ml)	25-49 nmol/L	-	50-74 nmol/L	>75 nmol/L
IOM[2]	<30 nmol/L (<12 ng/ml)	30-<50 nmol/L (12-<20 ng/ml)	≥50 nmol/L (≥20 ng/mL)	-	-
US Endocrine Society[3]	<50 nmol/L (<20 ng/mL)	52.5-72.5nmol/L (21-29 ng/ml)	75-250 nmol/L (30-100 ng/ml)	-	-

[1] IOF: International Osteoporosis Foundation
[2] IOM: Institute of Medicine
[3] US Endocrine Society Clinical Practice Guideline

Vitamin D deficiency is common and can occur at any age. Various cases of vitamin D deficiency have been seen in newborns (Sachan, et al. 2005), adolescents (Gordon, et al. 2004), pregnant women (Sachan, et al. 2005), healthy men and women (Heshmat, et al. 2008), and postmenopausal women (Suriah A Rahman, et al. 2004). The factors that contribute to vitamin D deficiency include race (Hannan, et al. 2008) (lighter skinned ethnic groups have higher serum 25(OH)D levels compared to darker-skinned ethnic groups living in the same geographical area) (Rockell, et al. 2008); skin pigmentation (melanin, the major natural pigment in the skin, interferes with cutaneous production of vitamin D and persons with darker skin colour are at increased risk of vitamin D insufficiency); age (increasing age reduces the ability of the skin to synthesize vitamin D); weight (higher adiposity has been correlated with lower vitamin D levels); avoidance of sun exposure and/or use of sunblock (due to voluntary non-exposure or for cultural reasons); malabsorption disorders such as celiac disease, Chron's disease and cystic fibrosis (affecting the body's ability to absorb vitamin D); disease and disorders of the kidneys and/or liver (affecting vitamin D metabolism); and use of medications such as anticonvulsants, anti-rejection medications and corticosteroids (Gozdzik, et al. 2008).

1.2 Statement of the Problem

Exposure to the sun is sufficient to produce adequate levels of vitamin D (Pedersen, 2008 & Whiting, et al. 2005). Pedersen (2008) reported that exposure of the face, arms, hands and legs to sunshine for 6-8 minutes for 2-3 times per week is more than sufficient to guarantee the requirement of vitamin D. Since the sun is the important element in improving vitamin D levels in the blood, people in tropical countries should have adequate levels of vitamin D in accordance with the hot and humid climate throughout the year. However, several researchers have claimed the prevalence of vitamin D deficiency/insufficiency in tropical countries such as Thailand (Chailurkit, et al. 2011), Vietnam (Ho-Pham, et al. 2011), India (Zargar, et al. 2007), Hawaii (Binkley, et al. 2007), Saudi Arabia (Sadat-Ali, et al. 2009) and Bangladesh (Islam, et al. 2008). Previous studies have also reported the discrepancies in vitamin D levels between Indians in urban and rural areas (Harinarayan, et al. 2008).

The scenario of vitamin D deficiency/insufficiency has also been seen in Malaysia (Suriah A Rahman, et al. 2004, Foong, 2011 & Foong, et al. 2011). The outcome of vitamin D deficiency such as bone mineral density has, however, not been well studied. In addition, previous reported studies (Green, et al. 2008; Khor, et al. 2011; Suriah A Rahman, et al. 2004; Foong, 2011 & Foong, et al. 2011) were conducted mainly in urban settings and did not measure sun exposure, skin colour, vitamin D and calcium intake. The present research was conducted to evaluate the vitamin D status in a large sample of participants involving those staying in urban and in rural areas. The present study also determines the factors that contribute to vitamin D levels among Malaysians.

1.3 Purpose

General Objective:

- To assess the vitamin D status of urban and rural women and its' correlates.

Specific Objectives:

- To determine the intake of vitamin D and calcium among ethnicities, age groups and urban and rural population in Malaysia and their association with serum vitamin D level.
- To compare the levels of sun exposure among the urban and rural population in Malaysia and to identify the correlation between sun exposure and 25(OH)D concentrations.
- To identify other factors that associated with vitamin D status. This includes age, race, socioeconomic status (SES), BMI, body fat percentage, skin colour, serum i-PTH levels and use of vitamin D containing supplements.
- To differentiate between the dietary vitamin D intake measured by vitamin D FFQ and 24-hour dietary recalls (24-HDRs) and to identify the correlation between them.

1.4 Significance of the Study

The current research could contribute to the findings of vitamin D status in a larger sample of the Malaysian population and gauge the extent of the problem and factors contributing to it so that effective measures can be taken to overcome this. The results

of the study could help the Ministry of Health or other agencies to develop a plan to improve the status of vitamin D such as educating Malaysians regarding the importance of vitamin D. In addition, the vitamin D and calcium food frequency questionnaire introduced to the subjects may increase their knowledge on vitamin D and calcium food sources.

1.5 Research Questions

The present research was conducted to answer the following questions:

1. What is the level of vitamin D amongst women?
2. To know the dietary intake of vitamin D and vitamin D food sources. Do the dietary intakes differ between area (urban and rural), ethnicities and age groups? Does the dietary intake of vitamin D and calcium have any influence on vitamin D levels?
3. Does the sun exposure differ between urban and rural women? Is correlation exists between sun exposure and vitamin D levels?
4. What other factors correlate with vitamin D level? The factors measured are:
   - Age
   - Race
   - SES (Socioeconomic Status)
   - BMI and body fat percentage
   - Skin colour
   - i-PTH levels
   - Vitamin D containing supplements
5. Do the above factors differ between urban and rural women?

6. Is there a significance difference between dietary vitamin D intake measured by FFQ and 24-HDRs and does a correlation exist between them?

1.6 Hypothesis

Related to general objective:

1. Research Hypothesis (H_a): Urban women in Malaysia have lower levels of serum 25(OH)D compared to rural women.

   Null Hypothesis (H_0): Urban women in Malaysia have higher levels of serum 25(OH)D compared to rural women.

Related to specific objective (1):

1. H_a: Malay women have a higher dietary intake of Vitamin D and calcium compared to Chinese and Indian women.

   H_0: Malay women have a lower dietary intake of Vitamin D and calcium compared to Chinese and Indian women.

2. H_a: Older women have a lower dietary intake of vitamin D and calcium than younger women.

   H_0: Older women have a higher dietary intake of vitamin D and calcium than younger women.

3. H_a: Urban women in Malaysia have a lower dietary intake of vitamin D and calcium compared to rural women.

   H_0: Urban women in Malaysia have a higher dietary intake of vitamin D and calcium compared to rural women.

4. H_a: Increase of vitamin D and calcium consumption were significantly associated with increased serum 25(OH)D among/in Malaysian women.

H_0: Increase of vitamin D and calcium consumption were not significantly associated with increased serum 25(OH)D among/in Malaysian women.

Related to specific objective (2):

1. H_a: Urban women in Malaysia had lower sun exposure compared to rural women.

   H_0: Urban women in Malaysia had higher sun exposure compared to rural women.

2. H_a: Increased sun exposure was significantly associated with increased serum 25(OH)D.

   H_0: Increased sun exposure was not significantly associated with increased serum 25(OH)D.

Related to specific objective (3):

1. H_a: Increased age was significantly associated with decreased 25(OH)D.

   H_0: Increased age was not significantly associated with decreased 25(OH)D.

2. H_a: Malay women had higher vitamin D levels compared to Chinese and Indian.

   H_0: Malay women had lower vitamin D levels compared to Chinese and Indian.

3. H_a: Increased SES was significantly associated with increased 25(OH)D.

   H_0: Increased SES was significantly associated with decreased 25(OH)D.

4. H_a: Increased BMI and body fat percentages was significantly associated with decreased 25(OH)D.

   H_0: Increased BMI and body fat percentages was not significantly associated with decreased 25(OH)D.

5. H_a: Lightest skin colour was positively associated with 25(OH)D levels.

   H_0: Lightest skin colour was negatively associated with 25(OH)D levels.

6. H_a: Decreased i-PTH was associated with increased 25(OH)D levels.

   H_0: Decreased i-PTH was associated with decreased 25(OH)D levels.

.7. H_a: Subjects who use of vitamin D containing supplements have higher 25(OH)D levels than subjects who did not use vitamin D containing supplements.

   H_0: Subjects who use of vitamin D containing supplements have lower 25(OH)D levels than subjects who did not use vitamin D containing supplements.

Related to specific objective (4):

1. H_a: Vitamin D intake derived from 24-HDRs were significantly lower than obtained from FFQ.

   H_0: Vitamin D intake derived from 24-HDRs were significantly higher than obtained from FFQ.

2. H_a: There was an association between vitamin D intake derived from FFQ and 24-HDRs.

   H_0: There was no association between vitamin D intake derived from FFQ and 24-HDRs.

CHAPTER II

2.0 LITERATURE REVIEW

2.1 Metabolism of Vitamin D

Photoproduction of vitamin D starts with the synthesis of the sterol provitamin D3 molecule 7-dehydrocholesterol. This is produced in large quantities in the skin and incorporated into plasma membrane lipid bilayers of cells in the dermis and epidermis. 7-dehydrocholesterol absorbs UVB radiation in the wavelength range 290–315 nm when the skin exposed to sunlight. The absorbed energy causes chemical bonds within the 7-dehydrocholesterol molecule to break and rearrange, resulting in the formation of previtamin D3 (Tsiaras, et al. 2011). Then, previtamin D3 is rapidly transformed to vitamin D3 by a heat-dependent process (Holick, 2007). The absorption of ultraviolet (UV) radiation was continued by previtamin D3 and vitamin D3 in a wide range of wavelengths (Tsiaras, et al. 2011). Excessive exposure to sunlight makes molecules previtamin D3 and vitamin D3 breakdown into inactive photoproducts (Holick, 2007).

Vitamin D2 is generated through the ultraviolet irradiation of a provitamin D sterol (ergosterol) in plants. The extra methyl group at C24 and a double bond at C22-23, make it differs from vitamin D3 (Wootton, et al. 2005). Vitamin D2 and vitamin D3 from food are integrated into chylomicrons and transported to the venous circulation by the lymphatic system. Vitamin D that is made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D–binding protein and transported to the liver. In the liver, vitamin D is converted to 25-hydroxyvitamin D [25(OH)D] by the enzymes vitamin D-25-

hydroxylase. Since 25(OH)D is biologically inactive (Holick, 2007) (except at very high, non-physiological levels) (Tsiaras, et al. 2011), it must be converted to the biologically active form, which is 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. This process occurs in the kidneys by the enzyme 25-hydroxyvitamin D-1α-hydroxylase (1-OHase) (Holick, 2007).

There are three mechanism involve in the production of 1,25$(OH)_2$D. One of the mechanism occurs in a feedback loop, whereby increased levels of 1,25$(OH)_2$D down-regulate synthesis of this metabolite by inhibiting the action of α_1-hydroxylase, and decreased levels have the opposite effect. Secondly, it occur in the hypocalcaemia condition, whereby hypocalcaemia stimulates the secretion of parathyroid hormone (PTH), which lead to the conversion of 25(OH)D to 1,25$(OH)_2$D by activating α_1-hydroxylase. Lastly, hypophosphatemia directly activates α_1-hydroxylase and hence increases formation of 1,25$(OH)_2$D (Kumar, et al. 2003).

2.2 Sources of Vitamin D

Vitamin D can be obtained from sun exposure, food (naturally or fortified food) and supplements (Burgaz, et al. 2007; Holick, 2007). Sunlight exposure is the major source of vitamin D (Green, et al. 2008). 250 µg (10,000 IU) of vitamin D3 were produced when an individual exposed to a single dose of UVB to the whole body (Barger-Lux, et al. 2002).

Vitamin D food sources keep 25(OH)D concentrations when sun exposure is limited, such as in winter months or lack of sun exposure. However, only a few foods naturally contain vitamin D. Vitamin D3 (cholecalciferol) normally comes from animal sources

such as fish, eggs and organ meats (Lee, et al. 2008). Fish that contain vitamin D3 is oily fish such as salmon (9 µg or 360 IU per 3.5-ounce serving), mackerel, and sardines. Egg yolks also contain vitamin D (≤1.25 µg or ≤50 IU per yolk). However, because of the cholesterol content of egg yolks, makes it a poor source of vitamin D (Holick, 2004). Vitamin D2 (ergocalciferol) normally comes from fungal sources such as mushrooms or yeast, depending on the type and sunlight exposure. Besides, there is also food that fortified with vitamin D such as milk and dairy products, which normally contain a small amount of vitamin D. Other fortified food is fatty spreads such as margarines, which normally obtained from plant oils (Lee, et al. 2008).

Food supplements such as cod liver oil and isolated vitamin D supplements are other sources of vitamin D (Lee, at al. 2008). Cod liver oil is a good source of vitamin D3 since it plays a vital role for bone health (Holick, 2004). Moreover, multivitamin preparations normally contain 10-25 µg/day (400-1000 IU/day) of vitamin D2 or vitamin D3 (Holick, et al. 2011).

The RDAs for vitamin D proposed by Institute of Medicine (IOM) in 2011 for individuals with age 1-70 and ≥71 years are 15 µg/day (600 IU/day) and 20 µg/day (800 IU/day) respectively, targeting serum 25(OH)D level of at least 50 nmol/L (20 ng/ml) which meet the requirements at least 97.5% of the population (Ross, et al. 2011). In addition, adults age 50 and above may need at least 37.5-50 µg/day (1500-2000 IU/day) of supplemental vitamin D to achieve vitamin D levels more than 75 nmol/L (30 ng/ml) (Holick, et al. 2011). However, as recommended by IOF, the vitamin D requirements will vary between individuals in terms of their baseline levels of 25(OH)D, sun exposure, BMI and other factors. For example, persons with regular effective expose to the sun may need vitamin D intake below 20 µg/day (800 IU/day). The vitamin D intake

among obese person, individuals with osteoporosis, limited sun exposure, malabsorption and non-European populations known to be at high risk for vitamin D deficiency may be need to be adjusted as much as 50 µg/day (2000 IU/day) (Dawson-Hughes, et al. 2010).

Mini-Summary

Vitamin D is obtained from sunlight exposure, vitamin D food and also supplements. Therefore, lack of sun exposure and inadequate intake of vitamin D food and supplements will lead to low serum vitamin D level. This condition can be prevented by educating the public through a campaign about the importance of sun exposure, vitamin D intake as well as food fortification with vitamin D.

2.3 Factors that Affects Vitamin D Status

2.3.1 Sun Exposure and Outdoor Physical Activity

Puri et al. (2008) observe the influence of sun exposure on vitamin D status in Delhi, India in healthy schoolgirls (6–18 years) which differ in socioeconomic status. Using Lips definition of hypovitaminosis D, 90.8% of the girls had hypovitaminosis D (<50 nmol/L). In the lower socioeconomic strata (LSES) groups, 89.6% of the girls had hypovitaminosis D [5.2% severe (<12.5 nmol/L), 25.4% moderate (12.5–25 nmol/L), 59% mild (25–50 nmol/L)], whereas 91.9% of the upper socioeconomic strata (USES) groups had hypovitaminosis D (2.8% severe, 36.5% moderate, 52.6% mild). The difference in serum vitamin D level can be explained by sun exposure where girls in the LSES groups had significantly higher sun exposure compared to USES girls. LSES

groups was found to spent more time in outdoor activities such as exercise, playing, and walking compared to USES girls (41.5±26.0 min/day vs 36.3±16.2 min/day, p=0.018). There was a significant correlation between serum 25(OH)D level and sun exposure (r=0.185, p=0.001), and between 25(OH)D and percentage of body surface area (BSA) (r=0.146, p=0.004).

According to Zargar et al. (2007), working as farmers gave a chance to expand the time and BSA exposed to the sun compared to other occupation. In addition, serum 25(OH)D concentrations were significantly related to sunlight exposure. Moreover, lower mean weekly exposure to the sun had been seen to those subjects with vitamin D deficiency compared to those who were vitamin D sufficient [15.6 (8.2) hour vs 20.6 (6.5) hour; p<0.03]. Furthermore, there was a significant difference in the mean of exposure to sunlight (hours per week) between subjects with and without vitamin D deficiency and among subjects with progressively severe vitamin D deficiency.

2.3.2 Intake of Vitamin D

Burgaz et al. (2007) that study the relationship between dietary intake of vitamin D and vitamin D intake from supplements to the serum concentration of 25(OH)D during winter (January–March) in 116 elderly women, aged 61-86 years old, living in central Sweden (latitude 60°) found that serum concentrations of 25(OH)D were significantly correlated with the dietary vitamin D intake (r=0.22, p=0.02). Moreover, those who took supplements regularly have 17% more serum 25(OH)D compared to those who did not on the supplements (78 and 67 nmol/L, respectively; p=0.06). The study also showed that 2–3 servings (130 g/wk) of fatty fish/wk increase 25.5 nmol/L of serum 25(OH)D concentrations. Moreover, daily intake of 300 g of vitamin D–fortified reduced-fat dairy

products increase 6.2 nmol/L serum 25(OH)D concentrations. In addition, regular use of vitamin D supplements increase 11.0 nmol/L of serum 25(OH)D concentrations.

Brock et al. (2007) wrote that dairy intake of less than a litre per week was significant predictors of vitamin D deficiency in Australian/British and Immigrant Vietnamese born elderly. He added that Australians elderly who consumed dietary vitamin D less than 1 µg/day (40 IU/day) appeared to be at a three-fold risk of deficiency [OR: 2.8 (0.4–21)]. 51% of immigrant Vietnamese elderly who live in Australia for an average of 20 years consumed more than 1 L of dairy a week compared to 73% of their Australian/British born counterparts; 58% had more than the Recommended Daily Allowance (RDA) of vitamin D (5 µg/day or 200 IU/day) compared to 70% of their Australian/British born counterparts; 53% more than the RDA of calcium compared to 81% of their Australian/British born counterparts.

Significant relationships were found between consumption of vitamin D food and vitamin D deficiency. Intake of soft drinks, fruit juice, and iced tea were positively correlated with vitamin D deficiency. In addition, milk and cold cereal were inversely correlated with vitamin D deficiency. On the other hand, there was no significant correlation between vitamin D deficiency and consumption of yogurt, cheese, or ice cream (Gordon, et al. 2004).

2.3.3 Gender

Gannage-Yared et al. (2000) found that hypovitaminosis D [25(OH)D<30 nmol/L or <12 ng/ml] was more common in women than in men (83.9% vs. 48.5%). Similarly, severe hypovitaminosis D [25(OH)D<12.5 nmol/L or <5 ng/ml] was more prevalent in

women (41.5%), especially for those covered their head, arms, and legs compared with the non-covered women (61.8% vs. 23.5%).

Mallah et al. (2011) also found that healthy male Jordanian had significantly higher level of plasma 25(OH)D compared to females Jordanian (44.5 ±10 nmol/L vs 31.1 ± 12 nmol/L). Females Jordanian were further classified according to the styles of clothing, where the mean of 25(OH)D were 40.3 nmol/L for the Western style, 31.3 nmol/L for the Hijab and 28.5 nmol/L for the Niqab groups. Style of clothing can limit exposure to the sun and thus affect the vitamin D synthesis and status.

The prevalence of vitamin D insufficiency (25(OH)D<75 nmol/L or <30 ng/ml) in Vietnamese population aged 18–87 years was 20% in men and 46% in women. When using the cut of point below 50 nmol/L (20 ng/ml) as vitamin D deficiency, the prevalence becomes 1% in men and 3% in women. Moreover, only 2% (4/205) of men and none of the women had 25(OH)D above 150 nmol/L (60 ng/mL). The high prevalence of vitamin D deficiency in women compared to men may be because of women prefer to avoid sun exposure and their style of clothing is more cover up (Ho-Pham, et al. 2011).

2.3.4 Body Mass Index (BMI) / Total Body Fat Mass (TBF)

Suriah A Rahman et al. (2004) claimed that the lower vitamin D levels amongst Malay compared to Chinese postmenopausal women might be due to the differences in body fat mass between those groups of women. Malay had a significantly higher BMI (27.2 vs 23.8 kg/m^2) and fat mass (25.4 vs 20.2 kg) compared to Chinese women. The authors

also found a significant negative correlation between vitamin D status with BMI (r=-0.27) and body fat mass (r=-0.24).

Khor et al. (2011) wrote that 58.0%, 17.9%, and 16.4% of children in primary school had normal BMI, overweight and obese respectively. In addition, the proportions of obese boys were significantly higher compared to girls (25.0% vs 9.5%). Moreover, the prevalence of vitamin D insufficiency is high, which 70.4% of the children had 25(OH)D levels <50 nmol/L. 35.3% of children had vitamin D deficiency (≤37.5 nmol/L), whereas 37.1% of the children had insufficient level of vitamin D (> 37.5 to < 50.0 nmol/L). The writers also said that vitamin D status was significantly correlated with BMI in boy's group.

Arunabh et al. (2003) investigated the relationship between vitamin D concentrations and the body fat percentage among healthy lean to mildly obese women aged between 20-80 years. The researcher found that this group of women had a mean BMI of 24 kg/m^2 (range, 17–30 kg/m^2); TBF of 23.6±7.3 kg; and mean TBF percentage of 36.2 ±7.4%. In addition, the serum level of 25(OH)D was decrease with the increasing of the percentage of TBF. Moreover, the mean concentration of vitamin D in the TBF quartile group of less than 31%, 32–37%, 38–42% and >42% was 56.6 nmol/L, 52.6 nmol/L, 50.8 nmol/L and 44.2 nmol/L respectively. There was a significant difference of vitamin D levels between the highest and lowest quartile of TBF percentage (mean 44.2 vs.56.6 nmol/L, $p<0.01$).

2.3.5 Location

2.3.5.1 Regional Variation

Chailurkit et al. (2011) study the vitamin D status of 2,641 adults, aged 15 - 98 years residing in five different areas in Thailand. Lower 25(OH)D levels had been seen in subjects who live in Bangkok (mean value below the sufficient level, 75 nmol/L) compared to the other regions in Thailand. Subjects live in the northeastern parts had the highest mean value of serum 25(OH)D. Subjects residing inside the municipal areas in all parts of Thailand (excluding the northeastern part) had lower circulating of 25(OH)D compared to subjects residing outside the municipal areas (central: 73.5 ± 1.2 nmol/L vs 82.5 ± 1.7 nmol/L, $p<0.001$; north: 75.6 ± 1.9 nmol/L vs 83.3 ± 1.1 nmol/L, $p<0.001$; northeast: 81.3 ± 1.4 nmol/L vs 82.4 ± 0.9 nmol/L, $p=0.001$; south: 71.9 ± 1.1 nmol/L vs 80.1 ± 1.3 nmol/L, $p<0.001$). In municipal areas, subjects residing in Bangkok had significant lower 25(OH)D levels compared to the other parts of the country ($p<0.01$). The differences in vitamin D value in each region in Thailand may be due to the regional variations in religion. Since most of the residents in Southern Thailand were Muslim, their styles of clothing were more covered-up. Moreover, working in agricultural fields make Northern Thai residents spend more time under the sun.

2.3.5.2 Urban Vs Rural

Harinarayan et al. (2007) found that both men and women from rural area have higher concentrations of 25(OH)D compared to urban subjects ($p<0.001$). 44% of men and 70% of women in rural area had 25(OH)D deficient (<50 nmol/L or <20 ng/ml); 39.5%

of men and 29% of women in rural area had 25(OH)D insufficient (50-75 nmol/L or 20-30 ng/mL); and 16.5% of men and 1% of women in rural area had 25(OH)D sufficient (>75 nmol/L or >30 ng/ml). On the other hand, 62% of men and 75% of women in urban area had 25(OH)D deficient; 26% of men and 19% of women in urban area had 25(OH)D insufficient; and 12% of men and 6% of women in urban area had 25(OH)D sufficient. The researcher stated that working as agricultural laborers, dress code, and duration of exposure to sunlight for approximately 8 hours/day might be contributed to the low prevalence of 25(OH)D deficiencies in rural subjects.

Same goes to another study conducted by Harinarayan et al. (2008), who found that both rural males and females healthy adults had significantly higher levels of 25(OH)D ($p<0.001$) compared to urban subjects. However, both urban and rural children had low concentrations of 25(OH)D. He also reported that location (urban and rural) was not significantly associated with 25(OH)D levels in both boys and girls. Moreover, there was no significant association between urban and rural locations based on the vitamin D status (deficient, insufficient and sufficient).

2.3.6 Race

Race is another factor that influences vitamin D status. The disparities in vitamin D levels among different racial groups have been studied before by previous researcher. For example, Hannan et al. (2008) that study the vitamin D status of Black, Hispanic and White men aged between 30-79 years found that White men (93.5 ± 35 nmol/L or 37.4 ± 14.0 ng/ml) had higher levels of 25(OH)D compared to Black (62.5 ± 36.8 nmol/L or 25.0 ± 14.7 ng/ml) and Hispanic (82.3 ± 34.8 nmol/L or 32.9 ± 13.9 ng/ml) men. In another study conducted in Black and White women and neonates showed that

higher percentages of Black women at delivery (29.2%) and neonates (45.6%) had vitamin D deficiency [25(OH)D<37.5 nmol/L] compared to 5% of White women and 9.7% of neonates. Higher percentages of Vitamin D insufficiency [25(OH)D between 37.5–80 nmol/L] also had been seen in Black women (54.1%) than White women (42.1%) (Bodnar, et al. 2007).

Besides Black and White populations, racial differences in vitamin D status also can be observed in Malay, Chinese and Indian subjects. For instance, Suriah A Rahman et al. (2004) reported that postmenopausal Malay women had significantly lower level of 25(OH)D compared to postmenopausal Chinese women (44.4 ± 10.6 nmol/L vs 68.8 ± 15.7 nmol/L, $p<0.05$). Furthermore, 88% of Chinese women compared to 27% of Malay women had hypovitaminosis D [25(OH) vitamin D concentrations between 50-100 nmol/L]. Besides, 12% of Chinese women compared to 71% of Malay women had vitamin D insufficiency [25(OH)D concentrations between 25-50 nmol/L]. The writers assumed that the higher melanin contents in Malay women compared to Chinese women increased the possibility of Malay women to get vitamin D deficiency.

2.3.7 Season

The seasonal variation in serum vitamin D levels have been reported in previous studies. This variation might be due to the total sunlight radiation reaching the skin in summer and winter months (Barger-Lux, et al. 2002). In Japan, serum 25(OH)D was higher in summer compared to late autumn (68.5 vs 53.5 nmol/L or 27.4 vs 21.4 ng/ml). The prevalence of vitamin D deficiency also is higher in late autumn (46.7%) than summer (9.3%) (Nanri, et al. 2011). Another study conducted by Barger-Lux et al. (2002) stated

that serum 25(OH)D was significantly higher in late summer compared to late winter (122 vs 74 nmol/L, $p<0.0001$).

2.3.8 Age

The older persons have higher risks to get vitamin D deficiency. This is because aging decrease the production of vitamin D by the skin, less sun exposure, and poor dietary consumption of vitamin D (Lee, et al. 2008; Aldasouqi, et al. 2011; Kruavit, et al. 2012). For instance, higher percentage of older (≥50 years old) (37%) Saudi Arabia men had low vitamin D levels compared to younger men (25-35 years old) (28%). On the other hand, 72% and 63% of younger and older men had normal vitamin D (≥75 nmol/L or ≥30 ng/ml) respectively (Sadat-Ali, 2009). Kruavit et al. (2012) that study the vitamin D status of the elderly Thai staying in nursing home reported that 25(OH)D levels of his subjects (65 nmol/L) was lower than the previous study conducted in younger Thai women (73–129 nmol/L).

Although most study reported the lower vitamin D levels with advancing age, this is in contrast with finding by Chailurkit et al. (2011) who found that vitamin D levels is higher with increasing age and younger age becomes predictors of vitamin D inadequacy in Thailand population. The writers assumed that increase sun exposure might increase the vitamin D levels of older person. On the other hand, increase application of sunscreen contributed to the lower vitamin D levels of younger population in Thailand.

Mini-Summary

Various factors influence vitamin D levels, some of which include sun exposure, vitamin D intake, gender, BMI, location, race, season, and age. Previous studies have shown different association between each factor and the level of vitamin D. Hence, further study is required to determine the function of each of the factor in improving vitamin D levels.

2.4 Assessment of Vitamin D status

2.4.1 Sun Exposure Assessment

There are several methods to measure sun exposure such as questionnaire and dosimeter badges. For example, Sahota et al. (2008) used questions regarding sun exposure during the previous four weeks in both summer and winter time administered to the subjects via telephone interview. The subjects were asked regarding their outdoor job (working outdoors between 9 a.m. and 5 p.m.; classified as ‘‘yes’’ or ‘‘no’’), days outside per week (where at least 0.5 hour each day was spent outdoors; classified as ‘‘<7’’ or ‘‘7’’), limb coverage (coverage of arms and legs; classified as ‘‘yes,’’ ‘‘partial,’’ or ‘‘no’’), and sunscreen use (classified as ‘‘yes’’ or ‘‘no’’) during summer season. In winter time, subjects were also need to answer the questions pertaining to the trip to a warm, sunny climate in the winter (classified as ‘‘yes’’ or ‘‘no’’), limb coverage while on trip (coverage of arms and legs; classified as ‘‘yes,’’ ‘‘partial,’’ or ‘‘no’’), and sunscreen use while on trip (classified as ‘‘did not go on trip,’’ ‘‘yes,’’ or ‘‘no’’).

Other study conducted by Hall et al. (2010) used polysulphone (PS) dosimeter badges as a tool to measure sun exposure. All subjects were supplied with PS badges and worn this badges on their right wrist by attaching it to a white sweat band. The badges were worn by all subjects once per week on the same day from 7.00 am to 7.00 pm. The time spending outside including time of day, venue, outdoor activity, time in the direct sun or shade, use of sunscreen, and attire were recorded by the subjects. Individual dose of sun exposure (J) were calculated based on multiplication of the daily PS badge measurement (J/m^2) and mean m^2 of exposed skin when participants were in the sun.

The interview session was conducted by Barger-Lux et al. (2002) in order to study the effects of summer sun exposure on vitamin D levels. The respondents consist of 30 healthy men who had expanded their outdoor activity in a summer season. The duration of outdoor activity, usual weekly activity, sunscreen application and usual outdoor attire were recorded. In order to determine the usual skin exposure, the rule of nine was adapted to illustrate the body surface are (BSA) which include combination of shirt, pants and hat. The researcher also calculate the sun index which combining a measure of length of outdoors activities during daytime and BSA usually exposed during that time, where sun index = hours of sun exposure per week x fraction of BSA exposed to the sun. To calculate the skin tone of sun-exposed areas, a cosmetic colour chart was applied on a nine-point ordinal scale ranging from lightest to darkest (*i.e.* 0–8), with half-point values for intermediate readings.

2.4.2 Vitamin D Intake Assessment

There are several methods to assess dietary vitamin D intake. This includes food frequency questionnaire, dietary recalls and weighed food record.

The interview session regarding vitamin D intake was conducted in US adults, aged ≥20 years in order to determine the relationship between vitamin D intake and 25(OH)D concentrations. The respondents were asked about the frequency and type of food intake over the past month. The vitamin D food consist of breakfast cereal, generally fortified with 1-1.25 μg (40–50 IU) of vitamin D per 28-g serving, covered the major brands for cold cereals plus cooked, hot cereals; milk and vitamin D supplements. The frequency of taking the food was categorized into 0, 1-12, 13-30 and ≥31 times in the last month. In addition, information regarding consumption of vitamins or minerals in the past month was also obtained from the subjects. The subjects were asked to bring along the supplements later at the mobile examination centers for the investigator to check the contents of the supplement (Scragg, et al. 2008).

Two sources of vitamin D food were assessed by Lym et al. (2009) in his study involving Korea men aged 40–69 years. It includes dark fish (i.e. mackerel, Spanish mackerel, saury, tuna, and salmon) and dairy foods (i.e. milk, yogurt, cheese and ice cream). The fish listed in his questionnaire regularly consumed by Korean people and contain high level of vitamin D (mackerel: 11 μg of vitamin D/100 g, Spanish mackerel: 12 μg/100 g, saury: 16 μg /100 g, tuna 12 μg/100 g, salmon 32 μg/100 g). The respondents were asked whether they eat the food <1/week, 1-2/week, or >3 times/week.

2.4.3 Kits for the Assessment of Vitamin D Concentrations

Various commercial kit assays are designed for the assessment of vitamin D concentrations (Wootton, et al. 2005). This includes Radioimmunoassay (RIA), High-

Performance Liquid Chromatography (HPLC), Liquid Chromatography-Mass Spectrometry (LC-MS) and automated immunoassay (Wagner, et al. 2009).

2.4.3.1 Radioimmunoassay (RIA)

RIA can measured both 25(OH)D2 and 25(OH)D3 (Leino, et al. 2008). The disadvantage of RIA is it is affected by matrix effects (Barake, et al. 2012) such as lipid or anything present in the samples which reduce the capability of the binding agent, antibody, or binding protein to attach with 25(OH)D in the sample or standard (Hollis, 2007; Barake, et al. 2012). Binkley et al. (2007) that measure the vitamin D concentrations using reverse-phase HPLC and RIA claimed the high correlation between both methods (r^2=0.76). However, a systematic bias was found whereby the value of 25(OH)D measured by RIA was higher by 17 nmol/L (6.8 ng/ml) than HPLC. As a consequences, low prevalence of subjects have low vitamin D status [25(OH)D cut-off of 75 nmol/L or 30 ng/ml] determined by Diasorin RIA.

Paul et al. (2008) determine 25(OH)D concentrations by using a radioimmunoassay (DiaSorin, Stillwater, Minnesota). The analytical sensitivity of this assay, which is the lowest quantity differentiated from zero at 2 SD above the mean counts per minute of the zero calibrator (N = 20), is 3.75 nmol/L (1.5 ng/ml). The normal range of 25(OH)D for this kits is 22.5 to 94 nmol/L (9.0 to 37.6 ng/ml). The intra-assay coefficient of variation (CV) is 5.5% at a vitamin D level of 39 nmol/L (15.6 ng/mL) and 9.3% at a vitamin D level of 131.3 nmol/L (52.5 ng/ml).

2.4.3.2 High-Performance Liquid Chromatography (HPLC)

High-Performance Liquid Chromatography use direct detection methods for analyzing circulating of 25(OH)D. This method is considered as the gold standard method for measuring vitamin D. It separate and quantitate circulating of 25(OH)D2 and 25(OH)D3 individually. The disadvantages of this technique are burden, need many sample and radioactive internal standard and require a well trained technician (Hollis, et al. 2008).

2.4.3.3 Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid Chromatography-Mass Spectrometry also can be used for measurement of 25(OH)D levels. It also quantitates 25(OH)D2 and 25(OH)D3 separately. LC-MS is an accurate testing method if conduct in appropriate manner. Nevertheless, the tools is costly and its overall sample throughput cannot match that of the automated instrumentation format. The disadvantages of this techniques is it is unable to distinguish between 25(OH)D3 and its inactive isomer 3-epi-25(OH)D3 (Hollis, et al. 2008), resulting in overestimation of serum 25(OH)D levels compared to the actual value (Holick, et al. 2005). Therefore, lower prevalence of vitamin D insufficiency was measured by this technique (Holick, et al. 2005). In addition, it needs an internal standard for each compound quantitated and a trained technologist to work with the system (Hollis, et al. 2008).

2.4.3.4 Competitive Chemiluminescence Assay

Competitive Chemiluminescence Assay provide a direct (no extraction) quantitative measurement of serum or plasma 25(OH)D. This includes DiaSorin Corporation, Roche Diagnostics and Nichols Institute Diagnostics (Hollis, 2008).

Nichols Diagnostics launched the fully automated chemiluminescence Advantage 25(OH)D Assay System in 2001. The un-extracted serum or plasma will be added to a mixture of human DBP, acridinium-ester-labeled anti-DBP, and 25(OH)D3-coated magnetic particles in this assay. However, it tends to overestimated total vitamin D concentrations and also unable to determine 25(OH)D2, resulting in its removal from the market in 2006 (Hollis, 2008).

DiaSorin Corporation commenced the fully automated chemiluminescence Liaison 25(OH)D Assay System in 2004. Since it is co-specific for 25(OH)D2 and 25(OH)D3, hence it is able to measure a total 25(OH)D levels (Hollis, 2008).

In 2007, Roche Diagnostics released an automated 25(OH)D assay, called Vitamin D3 (25-OH) which can be analyzed on the Roche Elecsys or Cobas System (Hollis, 2008). The method applied a principle of competitive assay, whereby the binding protein of vitamin D is inactivated during incubation. This method use a polyclonal antibody directed against 25(OH) vitamin D3. The assay only able to determined 25(OH)D3, but not vitamin D2. Hence, this assay may underestimate the vitamin D status in those individuals who was on vitamin D2 supplement (Leino, et al. 2008).

Wagner et al. (2009) evaluate the analytical performance of two automated chemiluminescence-based immunoassays, which are LIAISON 25 OH Vitamin D TOTAL and Roche ECLIA Vitamin D3 (25-OH) assay with the reference method, which is DiaSorin 25(OH)D RIA for the assessment of serum 25(OH)D. He reported that LIAISON showed a stronger correlation and better agreement (r=0.918, bias=−0.88 nmol/L) with the reference method (DiaSorin RIA) compared to the Roche 25(OH)D3 assay (r=0.871, bias=−2.55 nmol/L). The lower correlation and agreement of the Roche assay with the reference assay is probably due to the assay itself. Roche 25(OH)D assay is more likely to overestimate 25(OH)D at low concentrations (<40–50 nmol/L) and underestimate 25(OH)D at high concentrations (>75–100 nmol/L).

Another study conducted by Leino, et al (2008) compare the analytical performance of the Roche Elecsys 25(OH)D3 assay with a manual RIA method which is DiaSorin 25(OH)D RIA, an HPLC method with ultraviolet detection and a liquid chromatography–tandem mass spectrometry (LC-MS/MS) assay. He claimed that 25(OH)D3 levels evaluated by the Roche Elecsys were in good overall agreement with the results illustrated by LC-MS/MS and RIA.

Mini-Summary

Measuring vitamin D levels can be achieved using various methods and commercial kit such as RIA, HPLC, LC-MS and automated immunoassay. Each commercial kit has advantage and disadvantages. Since the variability of the results obtained from the kits and laboratories may affect the status of vitamin D, hence, vitamin D assays and lab performance should be standardized and improved for better assessment of vitamin D status.

2.5 Vitamin D Cut-off Points

Various cut-off points were used by previous authors to define vitamin D deficiency, insufficiency and sufficiency. For instance, Hintzpeter et al. (2008) used 25(OH)D cut-off points as following: severe vitamin D deficiency (<12.5 nmol/L), moderate vitamin D deficiency (12.5–25 nmol/L), mild vitamin D deficiency (25–50 nmol/L), and safe reference limit (>50 nmol/L).

In contrast, Harinarayan (2005) categorized serum 25(OH) D levels according to the functional health–based values: (1) normal vitamin D, 25(OH)D greater than 50 nmol/L or 20 ng/ml; (2) vitamin D insufficiency (hypovitaminosis D), 25(OH)D between 25 and 50 nmol/L (between 10 and 20 ng/ml); and (3) vitamin D deficiency, 25(OH)D less than 25 nmol/L or 10 ng/ml [moderate vitamin D deficiency, 25(OH)D between 12.5 and 25 nmol/L (between 5 and 10 ng/ml), and severe vitamin D deficiency, 25(OH)D less than 12.5 nmol/L or <5 ng/ml].

Besides, Gordon et al. (2004) illustrated 25(OH)D concentrations ≤50 nmol/L (≤20 ng/ml) as vitamin D insufficiency; ≤37.5 nmol/L (≤15 ng/ml) as vitamin D deficiency and (≤20 nmol/L (≤8 ng/ml) as severe vitamin D deficiency. The definition of vitamin D deficiency was based on previous literature that reported patients with vitamin D levels of ≤37.5 nmol/L (≤15 ng/mL) had high PTH levels. On the other hand, the definition of severe vitamin D deficiency was based on the assay threshold (22.5 nmol/L or 9 ng/ml) for 25(OH)D, according to the normal range of the Nichols Institute.

Mini-summary

A variety of approaches have been described in the literature to develop vitamin D cut-off points. 25(OH)D level >50 or 75 nmol/L, for examples are widely use to determine vitamin D sufficiency. Some researcher use functional health–based values to classified vitamin D status, while the others look at the relationship between 25(OH)D and PTH levels for the same reason. It is difficult to define vitamin D deficiency/insufficiency based on a single cut-off points since the effects of vitamin D in individual living in a different geographical area are inconsistent. Therefore, appropriate cut-off points of vitamin D status should be studied in depth.

2.6 Prevalence of Vitamin D Deficiency/Insufficiency

Prevalence of vitamin D deficiency/insufficiency among children and adolescents was reported earlier. As an example, Hintzpeter et al. (2008) that conducted a study on 25(OH)D concentrations of 10,015 children and adolescents, aged 1–17 years, who participated in the German National Health Interview and Examination Survey for Children and Adolescents found that among 3 to 17 years old participants, 29% of immigrant boys and 31% of immigrant girls had severe to moderate vitamin D deficiency [25(OH)D concentrations <25 nmol/L] compared with 18% of non-immigrant boys and 17% of nonimmigrant girls. In addition, 92% of immigrant boys and 94% of immigrant girls had 25(OH)D concentrations <75 nmol/L (levels above 75 nmol/L are defined as optimal regarding various health outcomes) compared with 87% of non-immigrants. They also indicated that boys with a Turkish or Arab-Islamic background had an increased risk of having 25(OH)D concentrations <25 nmol/L compared with non-immigrants (odds ratio [OR] 2.3; [95% CI] 1.4–3.8 and OR 7.6;

[95% CI] 3.0–19.1). Similarly, girls with a Turkish (OR 5.2; [95% CI] 2.9–9.6), Arab-Islamic (OR 5.9; [95% CI] 2.5–14.0), Asian (OR 6.7; [95% CI] 2.2–19.8), or African (OR 7.8; [95% CI] 1.5–40.8) background had an increased risk of having severe to moderate vitamin D deficiency.

Another study conducted by Foo et al. (2009) that assess vitamin D deficiency in three different level of 25(OH)D concentrations, i.e. <25, <37.5 and <50 nmol/L reported that 32.8% of Chinese adolescent girls in Beijing, China had 25(OH)D level below than 25 nmol/L, 68.4% had 25(OH)D level below than 37.5 nmol/L and 89.2% had 25(OH)D level below than 50 nmol/L.

Among 51 healthy adolescent schoolgirls attending year 10 of an inner city multiethnic girls' school, 37 (73%) girls had vitamin D deficiency [serum 25(OH)D <30 nmol/L] and 9 (17%) had severely deficient [serum 25(OH)D<12.5 nmol/L]. In addition, the median of 25(OH)D concentration of white girls was significantly higher compared to the non-white girls ($p<0.001$) (Das, et al. 2006).

There were no exception for healthy men and women to get vitamin D deficiency/insufficiency. Such a study by Heshmat et al. (2008) that evaluated the prevalence of vitamin D deficiency in healthy men and women Iranian aged 20 to 69 years in five urban metropolitans' cities (Tehran, Tabriz, Mashhad, Shiraz and Booshehr). He reported that almost half of Iranian males and females had moderate to severe vitamin D deficiency. Moreover, men residing in Tehran and Mashhad had the highest prevalence of moderate to severe vitamin D deficiency. 66.0%, 60.2%, and 58.5% of male residing in Tehran aged less than 50 years, more than 60 years, and between 50 and 60 years respectively have moderate to severe vitamin D deficiency. On

the other hand, men and women residing in Booshehr had the lowest prevalence of moderate to severe vitamin D deficiency (men: 16.7% aged <50 years, 8.5% aged 50-60 years and 21% aged ≤60 years; women: 33.1%, 24.4% and 20.5%,). Besides, Tehran's women aged less than 50 years old (73.7%) and between 50 and 60 years old (51.4%) had the highest prevalence of moderate to severe vitamin D deficiency. Conversely, women aged >60 years residing in Mashhad had the highest prevalence of moderate to severe vitamin D deficiency.

Vitamin D deficiency/insufficiency also happens in postmenopausal women. Using population-based reference values, 126 of 164 postmenopausal south Indian women (77%) had normal 25(OH)D levels [22.5-94 nmol/L (9–37.6 ng/ml)], and 38 (23%) had 25(OH)D deficiency. On the other hand, by using functional health-based reference values, 30 (18%) patients had normal 25(OH)D levels [>50 nmol/L (>20 ng/ml)], 85 (52%) had 25(OH)D insufficiency [25-50 nmol/L (10–20 ng/ml)], and 49(30%) had 25(OH)D deficiency [<25 nmol/L (<10 ng/ml)] (Harinarayan, 2005).

The prevalence of vitamin D deficiency also had been seen in Malaysian children, adults and postmenopausal women. Khor et al. (2008) that examined the vitamin D status among primary school children in Malaysia aged between 7-12 years reported a high prevalence (70.4%) of vitamin D insufficiency [25(OH)D<50 nmol/L) among this children. In addition, girls (77.5%) had a higher prevalence of vitamin D insufficiency than boys (66.1%). A poor vitamin D status among children might be due to the low dietary intake and inadequate exposure to the sun.

Moy et al. (2011) found that only one third of 380 Malay workers in Kuala Lumpur had vitamin D sufficiency (≥50 nmol/L). Moreover, females (36.2 ± 13.4 nmol/L) had lower

concentrations of vitamin D compared to males (56.2 ± 18.9 nmol/L) workers. The possible explanation of the lower vitamin D levels among females than males workers were clothing styles which were more covered up, avoiding of sun exposure, use of umbrella and working indoors. This is shown by the low mean sun exposure score obtained by female compared to male workers (93.1 ± 14.6 vs 329.8 ± 54.3, $p<0.001$). Female workers also had a higher sun protection score than male workers (3.2 ± 0.9 vs 1.7 ± 0.7, $p<0.001$).

Other study conducted by Suriah A Rahman, et al. (2004) showed that Chinese had a higher levels of vitamin D than Malay postmenopausal women in Kuala Lumpur (68.8 ± 15.7 nmol/L vs 44.4 ± 10.6 nmol/L, $p<0.05$). She also reported that 27% Malay and 87% Chinese women had hypovitaminosis D [25(OH)D between 50-100 nmol/L). In addition, 71% Malay and 11% Chinese had vitamin D insufficiency [25(OH)D between 25-50 nmol/L).

Mini-summary

Vitamin D deficiency/insufficiency is common at all age categories including children and has been reported in many studies. However, comparing the prevalence of vitamin D deficiency in different countries among different population is impossible due to the different methods used in measuring the level of vitamin D as well as the variability of reported vitamin D cut-off points. Hence, further research is required to find more comparable literature in order to increase the information about vitamin D status in all population.

2.7 Vitamin D and Parathyroid Hormone (PTH)

In their research involving healthy Icelandic adult population, Steingrimsdottir et al. (2005) noticed that there was an inverse relationship between serum 25(OH)D and serum PTH levels. Those group who had sufficient serum 25(OH)D levels (value more than 45 nmol/L or >18 ng/ml) seem to have lowest serum PTH levels, whereas group who had serum 25(OH)D of less than 25 nmol/L (<10 ng/ml) have highest serum PTH. However, the relationship between serum 25(OH)D and serum PTH levels turn out to be statistically non-significant when the level of serum 25(OH)D was more than 45 nmol/L (>18 ng/ml). In addition, minor decrements in PTH levels were seen when the value of serum 25(OH)D was more than 45 nmol/L (>18 ng/ml).

2.8 Vitamin D and Health

2.8.1 Osteoporosis

Lips et al. (2006) determined the prevalence of vitamin D inadequacy in 2606 postmenopausal women with osteoporosis characterized by low bone mineral density and history of fragility fracture in 18 differences countries. He reported that the mean of serum 25(OH)D level was 67.0 nmol/L (26.8 ng/ml). Moreover, 63.9% of this women had serum 25(OH)D levels <75 nmol/L (<30 ng/ml). In addition, high prevalence (above 65%) of vitamin D inadequacy [25(OH)D levels <75 nmol/L (<30 ng/ml)] was seen in South Korea (92.1%), Japan (90.4%), Lebanon (84.9%), Turkey (76.7%), United Kingdom (74.5%), Germany (68.0%), Mexico (67.1%) and Spain (64.7%).

Another study conducted by Chee et al. (2010) in Chinese post-menopausal women in Kuala Lumpur aged between 50-70 years reported that subjects who have osteoporosis had lower vitamin D levels (58.3±8.15 nmol/L) compared to subjects who have osteopenia (60.4±16.1 nmol/L) or normal subjects (60.7±15.6 nmol/L).

2.8.2 Chronic Low Back Pain

Faraj et al. (2003) study the correlation between vitamin D deficiency and lower back pain among 360 patients, aged 15 to 52 years who had incident of low back pain for more than 6 months. 299 (83%) out of 360 patients have a low serum level of 25-OH vitamin D3.

2.8.3 Diabetes

Pittas et al. (2006) study the association of vitamin D and calcium intake with the risk of type II diabetes in women with no diagnosis of diabetes, heart disease, or cancer at the baseline. The subjects were trailed for 20 years. He found a significant inverse association between the intake of total vitamin D and possibility for developing type II diabetes. Women who took total vitamin D ≥20 μg/day (≥800 IU/day) had 23% in decreasing chances for developing diabetes compared with women who consumed <5 μg/day (<200 IU/day). In addition, women who consumed vitamin D from supplements of ≥10 μg/day (≥400 IU/day) have 13% lower risk to get diabetes than women who consumed ≤2.5μg/day (≤100 IU/day).

2.8.4 Cardiovascular Disease

The association between vitamin D deficiency and cardiovascular disease happen by several mechanisms. One experimental studies showed that 1,25-OH D participates in the renin-angiotensin axis regulation by directly suppressing renin gene expression. Wild-type mice can produce over expression of renin by pharmacological inhibition of vitamin D synthesis. Besides, receptors for vitamin D can be expressed by vascular smooth muscle cells and endothelial cells. Vascular smooth muscle cells and endothelial cells also have the ability to convert circulating 25(OH)D to 1,25-OH D. Vascular effects of vitamin D include modulation of smooth muscle cell proliferation, inflammation, and thrombosis. In addition, vitamin D deficiency causes secondary hyperparathyroidism. Parathyroid hormone (PTH) promotes myocyte hyper-trophy and vascular remodeling (Wang, et al. 2008).

The relationship between vitamin D status and cardiovascular disease was illustrated by Wang et al. (2008), using a subjects who had no history of cardiovascular disease at the baseline. The follow-up conducted in a mean 5.4 years later showed that 120 subjects had a first episode of cardiovascular disease. Moreover, subjects with high blood pressure and vitamin D deficiency have the highest rate of cardiovascular disease. He added that there is a strong association between 25(OH)D and cardiovascular risk among individuals with hypertension.

2.8.5 Hypertension

The links between vitamin D and hypertension occur with the influencing of vascular function and renin–angiotensin system. Moreover, 1α-hydroxylase enzyme that converts

25(OH)D to 1,25$(OH)_2$D is expressed in a variety of tissues, including human endothelial cells, human vascular smooth muscle cells (VSMC), and throughout the kidney. These data suggest a paracrine effect of 25(OH)D that is independent of circulating 1,25$(OH)_2$D levels, which challenges the traditional notion that biological activity of vitamin D is primarily dependent on conversion in the renal proximal tubule (Forman, et al. 2007).

Forman et al. (2007) investigated the association between 25(OH)D concentrations and high blood pressure in 613 men and 1198 women without history of high blood pressure. He reported that 61 out of 2282 men and 129 out of 4859 women developed hypertension in 4 years follow-up. 5.3% of person-years and 9.8% of cases had been seen in men who had plasma 25(OH)D levels <37.5 nmol/L (<15 ng/ml), while 6.9% of person-years and 8.5% of cases had been seen in women who had plasma 25(OH)D levels <37.5 nmol/L (<15 ng/ml). During 8 years follow-up, 133 cases out of 4243 person-years in men and 274 cases out of 7519 person-years in women develop hypertension. 5.3% of person-years and 6.7% of cases had been seen in men who had plasma 25(OH)D levels <37.5 nmol/L (<15 ng/ml), while 6.5% of person-years and 7.3% of cases had been seen in women who had plasma 25(OH)D levels <37.5 nmol/L (<15 ng/ml).

2.8.6 Cancer

Feskanich et al. (2004) look at the plasma 25(OH)D and 1,25$(OH)_2$D in a colorectal cancer cases and controls among women in the cohort of Nurses' Health Study (NHS). Mean plasma 25(OH)D was significantly lower (p=0.03) in women with colorectal cancer cases compared with controls among assayed in 2003 [67.5 vs 75.8 nmol/L (27.0

vs 30.3 ng/ml)], respectively but did not differ among assayed in 2000 [59.0 vs 60.8 nmol/L (23.6 vs24.3 ng/ml], respectively. On the other hand, $1,25(OH)_2D$ concentrations were same in the cases and controls for assayed in year 2000 and 2003. The researcher added that plasma 25(OH)D was inversely associated with risk of colorectal cancer.

2.8.7 Chronic Liver Disease

George et al. (2009) reported the high prevalence of vitamin D deficiency among patients with chronic liver disease (CLD). Moreover, 38 patients out of 63 patients (60%) had vitamin D value <25 nmol/L (<10 ng/ml). 20 patients (32%), had vitamin D value between 25-50 nmol/L (between 10-20 ng/ml) and 5 patients (8%) had vitamin D value >50 nmol/L (>20 ng/ml).

2.8.8 Chronic Kidney Disease (CKD)

Ali et al. (2009) wrote that the yearly mean level of 25(OH)D among children with CKD [between 11.6 to 30.2 ng/ml (29 to 75.5 nmol/L)] (n=79–336 per year). There were significant differences in the yearly mean vitamin D values over the decade. 20% to 75% of children with CKD had vitamin D deficiency [25(OH)D level <37.5 nmol/L (<15 ng/ml)], over the decade studied. Moreover, there were an increasing prevalence of vitamin D deficiency from the initial to the end of the decade ($p<0.001$).

Mini-Summary

Deficiency in vitamin D is associated not only with skeletal disorders, but also with non-skeletal diseases such as diabetes, heart disease and cancer. Other confounding factors like age and BMI might also contribute to vitamin D deficiency. Thus, a study evaluating the dose and the effects of vitamin D supplements on diseases should be conducted in a large sample size of population.

2.9 Research Framework

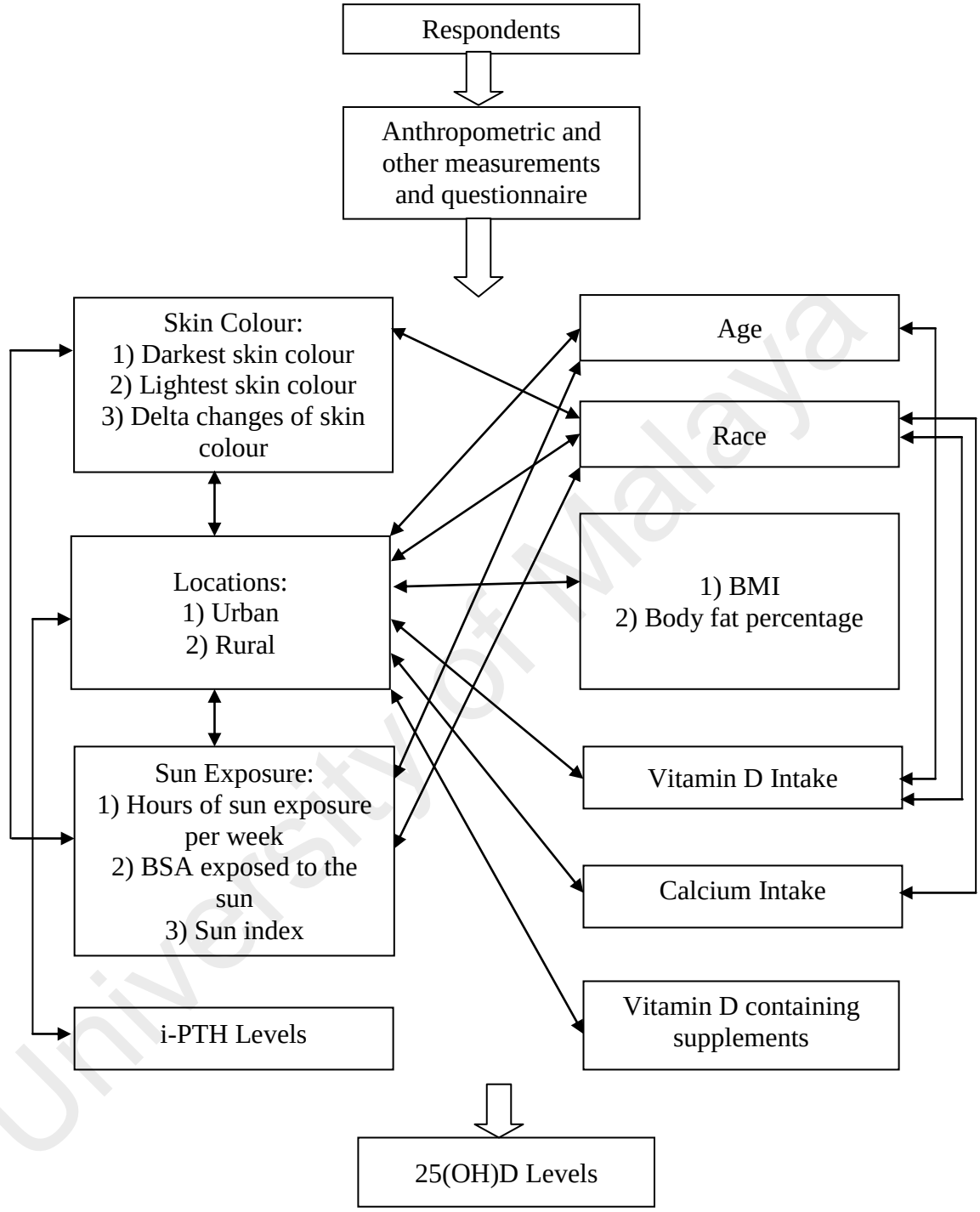


Figure 2.1 : Research Framework

CHAPTER III

3.0 METHODOLOGY OF STUDY

3.1 Population and Sampling

3.1.1 Study Population

Two groups of women (both urban and rural) aged above 45 years were recruited for the study. University of Malaya Medical Centre (UMMC), Lembah Pantai, Kuala Lumpur was chosen as a study area for the urban quota. At the same time, Palong 1 – 11, Pusat Bandar Palong, Negeri Sembilan was chosen as the rural study area. Urban women consisted of patients attending the outpatient clinics of UMMC. Rural subjects involved in the previous a 10-year ongoing follow-up research of non-communicable diseases in a rural Malaysian population for the year 2008-2010 were re-recruited. Most of urban subjects were recruited from gynecology clinic since the percentage of women attending this clinic is higher and hence easy to get as study subjects. Some of them also recruited from orthopedics clinics, where the probability of those who recommended for bone mineral density test is greater and hence easy to access as study participants.

A total of 419 rural women and 160 urban women were recalled to participate in this current research. A total of 126 rural women were excluded for the following reasons:

a) Having passed away

b) Having moved to a new location

c) No response to contact

d) Refused to participate; 53 of urban women were also excluded for similar reasons. A final count of 293 rural and 107 urban women co-operated and participated in this study.

Inclusion criteria involving women aged above 45 years old, dwelling in local community and able to look after themselves, urban: T-score for bone mineral density (BMD) test more than -2.5. On the other hand, women who had a history of osteoporosis, cancer, chronic liver failure and chronic kidney failure were excluded from this study.

54.2% and 50.9% of urban and rural women has at least one health problem (i.e. diabetes mellitus/hypertension/hyperlipidemia/ischaemic heart disease) respectively. For the urban population, subjects recruited were referred to the Nuclear Medicine Unit, UMMC for the BMD test. BMD was measured using Dual-Energy X-ray Absorptiometry (DEXA) (Machine: Lunar DPX-IQ) at the neck of femur and lumbar spine. WHO characterized BMD results as follow: a) normal: T-score >-1; b) osteopenia: T-score between -1 to -2.4; and c) osteoporosis: T-score ≤-2.5 (World Health Organization, 1994). According to this criteria, 74.8% and 66.4% of urban subjects had normal BMD at the neck of femur and spine L1-L4, respectively. Only 25.2% and 33.6% of them had osteopenia at the neck of femur and spine L1-L4, respectively. However, the BMD is not available in the rural area. Hence, the assessment of osteoporosis in rural area was based on the self-reported of previous history of osteoporosis. None of them reported they had osteoporosis.

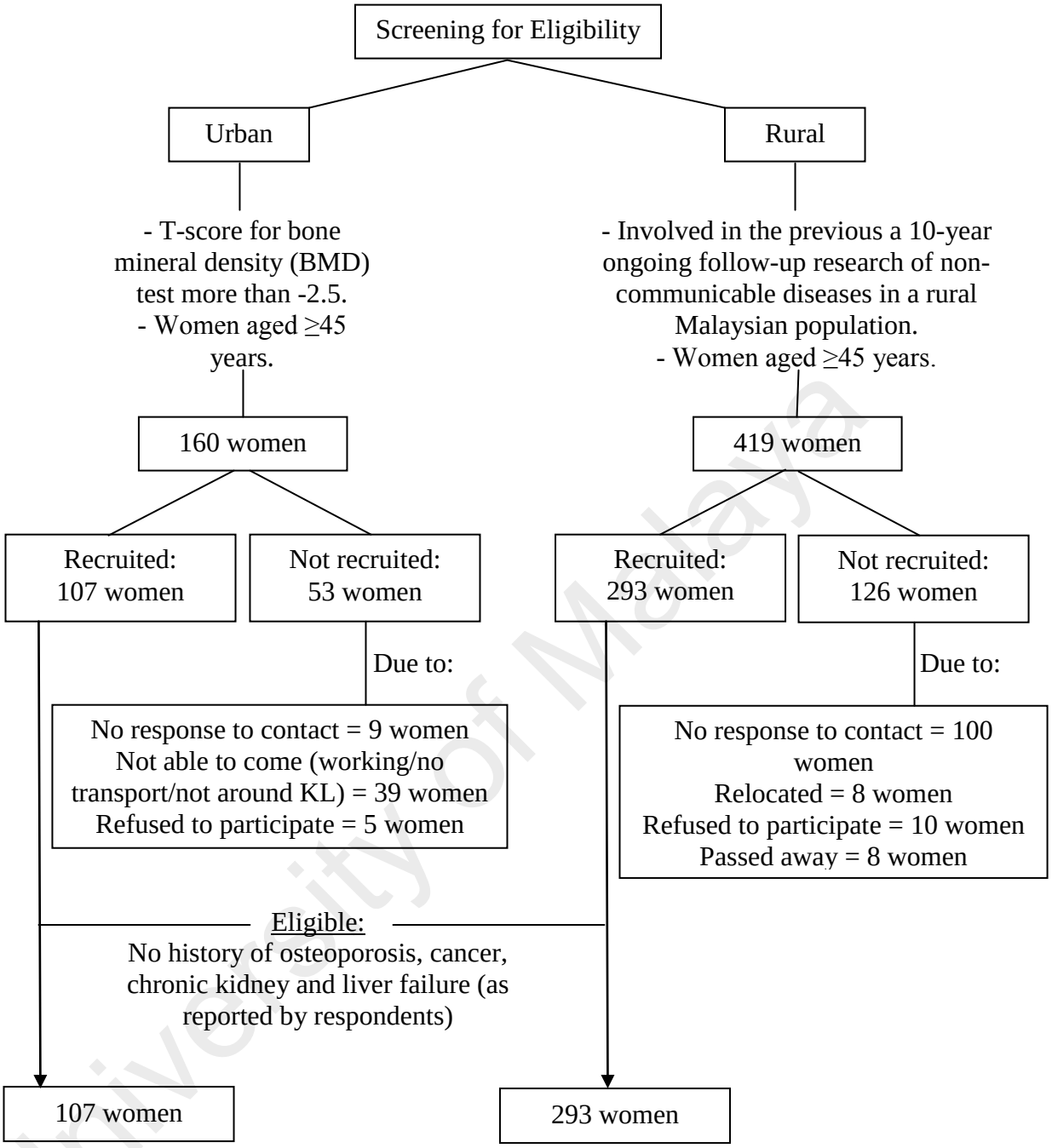


Figure 3.1 : Recruitment flow chart

3.1.2 Sample Size

The sample size was calculated based on the formula developed by Naing et al. (2006) and EPI Info 7:

3.1.2.1 Sample size calculation using formula

$$n = \frac{(Z_{1-\alpha/2})^2 \times p(1-p)}{e^2}$$

$$n = \frac{(1.96)^2 \times 0.733\ (0.267)}{0.05^2}$$

$$n = 301$$

Where,

n = required sample size

Z = 1.96 (confidence level of 95%)

p = prevalence: use 73.3% [based on the prevalence of vitamin D insufficiency obtained by Suriah A Rahman, et al. (2004)]

e = acceptable margin of error (5%)

3.1.2.2 Sample size calculation using EPI Info 7

A) <u>Urban</u>

The population size of urbanites was 25492. Using the prevalence of vitamin D insufficiency as 73.3% [Suriah A Rahman et al. (2004)] and a confidence limit of 5%, with confidence level of 95%, the sample size acquired in urban population was 297.

Population survey or descriptive study
For simple random sampling, leave design effect and clusters equal to 1.

Population size:	25492
Expected frequency:	73.3 %
Confidence limits:	5 %
Design effect:	1.0
Clusters:	1

Confidence Level	Cluster Size	Total Sample
80%	128	128
90%	210	210
95%	297	297
97%	363	363
99%	509	509
99.9%	820	820
99.99%	1133	1133

Figure 3.2 : Sample size calculation using EPI Info 7 (Urban)

B) <u>Rural</u>

The population size of the rural area was 26613. Using the prevalence of vitamin D insufficiency as 73.3% [Suriah A Rahman, et al. (2004)] and a confidence limit of 5%, with confidence level of 95%, the sample size required in rural population was 297 subjects.

Population survey or descriptive study
For simple random sampling, leave design effect and clusters equal to 1.

Population size:	26613
Expected frequency:	73.3 %
Confidence limits:	5 %
Design effect:	1.0
Clusters:	1

Confidence Level	Cluster Size	Total Sample
80%	128	128
90%	210	210
95%	297	297
97%	364	364
99%	509	509
99.9%	821	821
99.99%	1135	1135

Figure 3.3 : Sample size calculation using EPI Info 7 (Rural)

From the calculation, 297 respondents needed to be recruited from both urban and rural areas. Only 107 urban and 293 rural women were contactable and agreed to participate in this study. As stated above, only respondents who had been involved in the previous research were recalled. No data collection had been done to increase the number of respondents although the present research had insufficient sample size. This is due to

time constraints and the high expense incurred in data collection especially in the rural area.

3.2 Instrumentation

3.2.1 Questionnaire

One to one interviews based on the questionnaire were conducted. The questionnaire was prepared and was to be answered. The questionnaire consisted of six parts.

Part A: Demographic Details

Part B: Vitamin D Food Frequency Questionnaire

Part C: Calcium Food Frequency Questionnaire

Part D: Vitamin D-Containing Supplement

Part E: 24 Hour Dietary Recall and

Part F: Sun Exposure.

Face validity was conducted to identify any problems related to the questionnaire in terms of spelling errors, wording and time consuming to complete the questionnaire. Self-administrated questionnaire were distributed to 10 subjects. Most subjects took about 30 minutes in average to answer the questionnaire. This questionnaire was reviewed by a lecturer expertise in nutrition and clinical dietetics.

Part A: Demographic Details

The details of the subjects regarding age, gender, race, highest level of education completed, occupation, household income, and menopause status were collected.

Subjects were also asked whether they had ever discussed the importance of vitamin D to bone health; whether they had taken hormone replacement therapy; and a history of fracture.

Part B: Vitamin D Food Frequency Questionnaire (FFQ)

Vitamin D FFQ was developed to obtain vital information about vitamin D food intake. It consists of 29 foods that contain vitamin D. The questionnaire was developed using the methodology of measurement of FFQ constructed by Chee et al. (2002), in the research to compare the dietary calcium intake assessed by a semi-quantitative FFQ and a three-day food record method. Since there are no references to vitamin D content in the Nutrient Composition of Malaysian Food, food items on the market with vitamin D were listed and the vitamin D content was recorded. The mean of vitamin D in each similar type of vitamin D product was calculated to standardize the vitamin D content in each type of vitamin D product. The reading of each raw food by weight was adapted from the Nutrient Composition of Malaysian Food and United States Department of Agriculture (USDA) Standard Reference Database to determine the vitamin D content of the raw foods. Vitamin D food categories include milk and milk products (e.g. cheese, yogurt, butter, margarine); soy, beverages (e.g. chocolate beverages, nutritious malt drinks), cereals and products (e.g. bread, biscuits); fish and shellfish (e.g. pink salmon, sardines, mackerel, catfish); and meat products (e.g. eggs, liver).

The vitamin D food consumed by the subjects over the previous month was monitored. For commercial products, subjects were required to give the brand names of the product that they had taken. In addition, subjects were asked how often they ate or drank the foods and beverages daily, weekly or monthly; answers ‘never’ or ‘rarely’ for food that

were not commonly received. The subjects were also required to give the portion size of the food that they took whether small, medium, or large. Medium portion was used as the reference size as the portion normally taken by humans (medium = 1.0 × standard/medium portion size). A small portion is half of the medium portion (small = 0.5 × standard/medium portion size) and a large portion is one and half portions of the medium portion (large = 1.5 × standard/medium portion size).

Vitamin D, µg/day wascalculated for each food, where vitamin D, µg/day = vitamin D content of the food (according to portion size) x frequency of being consumed. Then, the sum of vitamin D intake was calculated.

Part C: Calcium Food Frequency Questionnaire (FFQ)

Calcium Food Frequency Questionnaire (FFQ) was obtained from FFQ that was developed by Chee et al. (2002) in the study to assess calcium intake in Chinese postmenopausal women in Kuala Lumpur. It consists of 78 food items that contributed to 95% of the total calcium intake of the population. The total calcium intake for the day was calculated by multiplying the consumption frequency, portion sizes and calcium content of the foods. Calcium food includes milk and milk products, vegetables, fruits, meat and products, fish and shellfish, cereals and products, legumes, tubers, mixed dishes, western fast food/kuehs and beverages (Chee, et al. 2002).

Part D: Vitamin D-Containing Supplement

Cod liver oil and other supplements taken by the subjects over the previous week were recorded. The frequency of taking the supplement and dosage of the supplement taken were also taken into account.

Part E: 24 Hour Dietary Recall (24-HDRs)

Food and drinks that had been consumed by subjects a day before the questionnaire was administered was recorded. The 24 Hours Dietary Recall was divided into five meal times, namely breakfast, lunch, tea, dinner and supper. For each meal, they needed to give the time they took the food and / or drinks, the name of the food and / or drinks, quantity of food and / or drinks and method of food preparation. The quantity of food was recorded in terms of household measurement namely bowls, cups, glasses, teaspoons and tablespoons. In addition, matchbox sizes were also used to illustrate the portion size of the food intake. Home recipes were also considered for uncommon dishes.

The weight of the food that was recorded in household measurement adapted from the Nutrient Composition of Malaysian Foods (Tee, et al. 1997). The unit is in grams. The nutrient content of each food was obtained from the Nutrient Composition of Malaysian Foods (Tee, et al. 1997). The recipes of the food that had not been listed in the Nutrient Composition of Malaysian Foods (Tee, et al. 1997) were obtained from the subjects or standardized recipes and calculated for nutrient content. The nutrient content of some commercial products that were not available in the Nutrient Composition of Malaysian Foods (Tee, et al. 1997) was obtained from the product label. In addition, Vitamin D

content of raw foods was obtained from the United States Department of Agriculture (USDA) Standard Reference Database (Nutritionist Pro™ Software, First DataBank, Inc., San Bruno, CA). The nutrient intake was analyzed using Nutritionist Pro™ Software (First DataBank, Inc., San Bruno, CA) (Axxya Systems LLC, 2009).

Part F: Sun Exposure

Sun exposure was assessed by inquiring about subjects's outdoor activities (under the sun), which include occupation, transport, activity at home, home repair, exercise/sports, and other activities over the previous week. Sunscreen use, time of the activity, duration of the activity, frequency of involvement in the activity for the previous week. The use of umbrellas and style of clothing worn during each activity reported by respondents was also recorded. The "Rule of Nine" was used to estimate the fraction of BSA exposed to sunlight by respondent's attire during outdoor activity, where attire included one of the selection of each category of attire (Janet, et al. 2002).

Table 3.1 : BSA exposed to sunlight

	Adapted "rule of nines"	Category 1			Category 2		Category 3	
		No shirt	Long-sleeved shirt	Short-sleeved shirt	Short pants	Long pants	No hat	Hat
Both arms	0.18	0.18	0.04	0.14				
Both legs	0.35				0.24	0.00		
Anterior trunk	0.18	0.09	0.00	0.00	0.00	0.00		
Posterior trunk	0.18	0.09	0.00	0.00	0.00	0.00		
Head	0.09						0.07	0.03
Perineum	0.01				0.00	0.00		
Column totals	1.00	0.36	0.04	0.14	0.24	0.00	0.07	0.03

"The "rule of nines" estimates sectors of adult BSA as percentages that are multiples of 9.

Sun index, which is an index combining a measure of time outdoors during daylight and BSA usually exposed during that time, was calculated as follows:

Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight (Janet, et al. 2002).

3.2.2 Anthropometric Measurement and Other Measurement

3.2.2.1 Height

The subject's height was measured using microtoise (seca model) to the nearest 0.1 cm. This scale was suspended on to the wall. The subjects were informed to remove their shoes and head accessories. The measuring bar of the scale was pushed up far enough to allow the person being measured to stand underneath it comfortably. The subject was asked to stand straight with heels together, back and head facing straight ahead. The measuring tongue was lowered until it came in contact with the head. The reading was then taken.

3.2.2.2 Body Mass Index (BMI)

Body mass index (BMI) is calculated as weight (in kg) divided by height (in m) squared.

3.2.2.3 Weight, Body Fat Percentage and Visceral Fat

Omron Body Composition Monitor with Scale Model HBF-362 Karada Scan was used to measure the weight, body fat percentage and visceral fat. This machine estimates the

body fat percentage based on the Bioelectrical Impedance Analysis (BIA) (Omron Package Insert). The subject's age, height and gender was entered into the machine before the measurement was taken. The subjects were then informed to remove their shoes, socks and things inside their pockets. Subjects were asked to step on the Karada Scan with their feet on the foot electrodes. In the mean time, they had to hold on to the grip of the electrodes of the Karada Scan with arms straight at a 90° angle until the measurement was complete.

3.2.2.4 Waist Circumference

The waist circumference was measured using measuring tape in centimeters (cms) to 1 decimal place. The subject was standing with feet shoulder width apart and arms crossed over the chest in a relaxed manner. The measurement was taken at the top of the iliac crest. The upper right hipbone was palpated until the uppermost lateral border of the iliac crest was found. The measuring tape was positioned in a horizontal plane around the abdomen.

3.2.2.5 Skin Colour Measurement

Skin colour was measured using a Cosmetic Colour Ruler (Skin Fairness Ruler - Garnier product) which has 16 colour points from 1 (lightest) to 16 (darkest). The darkest part of the body, which is on the back of the hand and lightest part of the body or natural skin colour which is under the arm was measured by this cosmetic ruler. The difference of highest and lowest points of the skin colour was calculated to determined delta skin colour or the changes of the skin colour (Binkley, et al. 2007).

3.2.3 Blood Analysis

A fasting blood sample (5 ml) was collected in a serum blood collection tube for serum 25(OH)D and i-PTH (intact-parathyroid hormone) analysis. The blood samples were kept at room temperature for 30 minutes to 1 hour to allow it toclot. Then, the sample was spun at 3500 revolutions per minute (RPM) for 15 minutes. The serum was separated into an aliquot tube and stored at -80°C until analysis. Two levels of control were run together with the samples during the analytical run of Vitamin D and i-PTH to confirm the reliability of the results.

3.2.3.1 Measurement of Vitamin D3 Level

Vitamin D level was analyzed using electrochemiluminescence immunoassay "ECLIA" Vitamin D3 (25-OH) method on the cobas e 411 analyzer. The assay was based on the competition principle. In the first incubation, the assay allows 25(OH) D3 in the sample to compete with biotin labeled vitamin D in the complex which contained biotin-vitamin D and monoclonal 25(OH) vitamin D3-specific ruthenium labeled 'antibody'. In the second incubation, streptavidin-coated microparticles were added, letting the complex bind to the solid phase by the interaction of biotin and streptavidin. Then, the microparticles were magnetically captured onto the surface of an electrode. A voltage was applied to the electrode to produce a chemiluminescent emission, which was measured by a photomultiplier [Wagner, et al. 2009; Vitamin D3 (25-OH) [package insert], 2010]. The measuring range for this kit is 10-250 nmol/L (4-100 ng/ml) (Vitamin D3 (25-OH) [package insert], 2010). The inter-assay CV at 57.0 nmol/L(22.8 ng/ml) and at 170.5 nmol/L (68.2 ng/ml) was 3.6% and 3.0% respectively. The intra-

assay CV at 57.0 nmol/L(22.8 ng/ml) and at 170.5 nmol/L (68.2 ng/ml) was 3.5% and 2.9% respectively.

This assay had been used previously for comparison with other assays analyzing the levels of vitamin D such as high performance liquid chromatography (HPLC) (Leino, et al. 2008), radioimmunoassay (RIA) and liquid chromatography–tandem mass spectrometry (LC-MS/MS), (Vitamin D3 (25-OH) [package insert], 2010; Leino, et al. 2008). The evaluation of the ECLIA assay with RIA and LC-MS/MS methods yielded an equation of Passing/Bablok of y=0.899x + 5.146 (r=0.859; n=125) and y=1.008x + 0.045 (r=0.902; n=771) respectively (Vitamin D3 (25-OH) [package insert], 2010). The assessment of the ECLIA assay with HPLC techniques yielded an equation of Elecsys = 1.077 x HPLC + 5.442 ($S_{y|x}$ = 13.9 nmol/L; n=67) (Leino, et al. 2008). This assay is unaffected by icterus (bilirubin <205 µmol/L), lipemia (intralipid <400 mg/dl) and biotin (<82 nmol/L) (Vitamin D3 (25-OH) [package insert], 2010).

3.2.3.2 Measurement of PTH Level

Serum i-PTH was analyzed using ECLIA PTH on the Cobas E-411 analyzer. The assay was based on the sandwich principle. In the first incubation, 50 µl of sample, a biotinylated monoclonal PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a ruthenium complex to form a sandwich complex. In the second incubation, the addition of streptavidin-coated microparticles caused the binding of the complex to the solid phase via interaction of biotin and streptavidin. Then, the reaction mixture was aspirated to the measuring cell where the microparticles were magnetically trapped onto the surface of the electrode. Unbound substances were removed with ProCell. Chemiluminescent emissions were induced with the application of a voltage to

the electrode. This emission was measured by a photomultiplier (PTH, intact [package insert], 2010).

The inter-assay CV at 2.14 pmol/L (20.2 pg/ml) and at 6.15 pmol/L (58.0 pg/ml) was 6.2% and 4.1% respectively. The intra-assay CV at 2.14 pmol/L(20.2 pg/ml) and at 6.15 pmol/L (58.0 pg/ml) was 4.1% and 2.2% respectively. The measuring range for this kit was 0.127-530 pmol/L (1.20-5000 pg/ml) (PTH, intact [package insert], 2010).

3.3 Procedure and Time Frame

3.3.1 Research Protocol

The research protocol which includes the respondent information sheet, consent form and questionnaire was approved by the Research Ethics Committee of University of Malaya Medical Centre [Medical Ethics Committee (MEC) reference no: 794.54 and 794.55]. The written informed consent was provided to the subjects before any of the study procedure was carried out.

3.3.2 Time Frame

Data for the urban sector was start collected on 21stAugust 2010. Subjects were informed about the study via phone. The appointment date, time and venue for a meeting with the researcher were arranged with the subject.

Data for the rural area was start collected on 25thOctober 2010. Permission from Federal Land Development Authority (FELDA) main office as well as a FELDA officer was

obtained before research was conducted at the rural area. Survey subjects were informed about the visiting date, time, and place by a letter sent to their home addresses.

3.3.3 Procedure

The respondent's information sheet and consent form were provided before the study was conducted. They were informed about the study by the researcher. Their initials and date were taken before the interview session and the necessary data regarding measurement was recorded.

3.4 Analysis Plan

The data were analyzed using PASW Statistical Package for Social Science (SPSS), Version 18.0. Categorical data was described using count and percentages. Continuous data was checked for normal distribution (Kolmogorov-Smirnov). Since it was not normally distributed, it was recorded as median and quartile 25-quartile 75 (Q25-Q75). The differences between population groups (urban vs rural) were evaluated using Chi-Square and Mann-Whitney U tests. The correlation between two continuous variables was analyzed using the Spearman correlation. The effects of independent predictors of vitamin D deficiency on serum 25(OH)D levels were explored using stepwise multiple linear regression analysis. A p-value of less than 0.05 was considered significant.

CHAPTER IV

4.0 RESULTS

4.1 Characteristics of subjects

Characteristics of the subjects are shown in Table 4.1. A total of 400 women participated in the current study, of which 107 women were from urban and 293 women were from rural areas respectively. The median age of the subjects was 57 years with a range of (53-61) years. Most of urban women were Chinese (52.3%), followed by Indians (28.0%) and Malays (19.6%). In contrast, rural subjects were predominantly Malays (84.0%), followed by Indians (12.3%) and Chinese (3.8%). All urban subjects were post-menopausal, whereas 21.8% and 78.2% of rural subjects were pre- and post-menopausal. Most of urban subjects had completed upper secondary school (50.9%) while the majority (67.6%) of the rural women had completed primary school only.

The majority of rural participants were housewives (82.6%) whilst 11.9% worked outdoors as rubber tappers or farmers. Most of the urban women were retired (45.8%). 29.0% were housewives and 25.2% working indoors. None of the urban women worked outdoors. In terms of household income, the urbanites draw significantly higher income compared to ther rural subjects (Table 4.1).

Table 4.1: Characteristics of subjects

	Overall, n=400	Urban, n=107	Rural, n=293	p-value
Age[a]	57 (53-61)	61 (58-65)	56 (52-59)	<0.001
Race[b]				<0.001
Malay	267 (66.8)	21 (19.6)	246 (84.0)	
Chinese	67 (16.8)	56 (52.3)	11 (3.8)	
Indian	66 (16.5)	30 (28.0)	36 (12.3)	
Education[b]				<0.001
No formal education	40 (10.0)	2 (1.9)	38 (13.0)	
Adult school	1 (0.3)	0 (0)	1 (0.3)	
Primary school	204 (51.1)	6 (5.7)	198 (67.6)	
Lower secondary school	51 (12.8)	9 (8.5)	42 (14.3)	
Upper secondary school	68 (17.0)	54 (50.9)	14 (4.8)	
Pre-University	5 (1.3)	5 (4.7)	0 (0)	
College/University	28 (7.0)	28 (26.4)	0 (0)	
Postgraduate degree	2 (0.5)	2 (1.9)	0 (0)	
Occupation[b]				<0.001
Working indoors	41 (10.3)	27 (25.2)	14 (4.8)	
Working outdoors	35 (8.8)	0 (0)	35 (11.9)	
Retired	51 (12.8)	49 (45.8)	2 (0.7)	
Housewife	273 (68.3)	31 (29.0)	242 (82.6)	
Household income, RM[a]	1500 (1000-2000)	3000 (1300-4000)	1400 (1000-1500)	<0.001
Menopausal status[b]				<0.001
Pre-menopause	64 (16.0)	0 (0)	64 (21.8)	
Post-menopause	336 (84.0)	107 (100.0)	229 (78.2)	

[a]Data as median (Q25-Q75)
[b]Data as n (%)

4.2 Physical and biochemical parameters

Table 4.2 shows the physical and biochemical parameters of the subjects. Rural women were significantly shorter than their urban counterparts. However there was no significant difference in their body weight, resulting in a higher median BMI. There was no significant difference in body fat percentages between urban and rural women, although rural women had significantly higher waist circumference and visceral fat compared to urban women.

Urban women had significantly lighter skin colour than rural women as can be shown by the mean skin colour scores. However, there was no significance difference in delta skin colour between the two groups of women (Table 4.2).

Table 4.2: Physical parameters

	Overall, n=400	Urban, n=107	Rural, n=293	p-value
Height (cm)[a]	152.0 (148.0-155.8)	153.8 (150.6-157.0)	151.0 (147.3-155.5)	<0.001
Weight (kg)[a]	62.6 (56.5-70.2)	61.9 (54.9-68.3)	62.7 (57.2-70.8)	0.125
BMI (kg/m^2)[a]	27.2 (24.9-30.1)	26.0 (23.1-29.0)	27.9 (25.2-30.5)	<0.001
Waist circumference(cm)[a]	93.0 (87.0-100.0)	90.0 (82.0-96.4)	94.0 (88.0-102.0)	<0.001
Body fat percentage (%)[a]	38.1 (35.7-40.6)	38.7 (36.0-41.2)	37.8 (35.5-40.4)	0.095
Visceral fat[a]	11 (8-14)	10 (7-13)	11 (8-15)	0.006
Darkest skin colour score[a]	12 (10-13)	9 (7-12)	12 (11-14)	<0.001
Lightest skin colour score[a]	5 (4-9)	4 (2-8)	6 (4-9)	<0.001
Delta skin colour[b]	5 (3-7)	5 (4-6)	6 (3-7)	0.138

[a] Data as median (Q25-Q75)
[b] Delta skin colour/the changes of the skin colour = Darkest skin colour – Lightest skin colour

4.3 Vitamin D and i-PTH status

The median levels of serum 25(OH)D and i-PTH of the subjects are presented in Table 4.3. Rural women had significantly higher levels of serum vitamin D (69.50 vs 31.90 nmol/L) and i-PTH (4.13 vs 3.59 pmol/L) than urban women.

Table 4.3: Biochemical parameters

	Overall, n=400	Urban, n=107	Rural, n=293	p-value
25(OH) D, nmol/L[a, b]	64.49 (45.58-75.34)	31.90 (26.05-45.50)	69.50 (58.95-79.14)	<0.001
PTH, pmol/L[a, c]	3.96 (3.08-5.26)	3.59 (2.78-4.79)	4.13 (3.14-5.51)	0.011

[a] Data as median (Q25-Q75)
[b]25(OH)D conversion factor: nmol/L x 0.40 = ng/ml (Vitamin D3 (25-OH) [package insert])
[c] PTH conversion factor: pmol/L × 9.43 = pg/ml (PTH, intact [package insert])

In Table 4.4, 25(OH)D levels were classified into <30 nmol/L (<12 ng/ml) as vitamin D deficient, 30-<50 nmol/L (12-<20 ng/ml) as vitamin D insufficient and ≥50 nmol/L (≥20 ng/ml) as vitamin D sufficient (Ross, et al. 2011; IOM, 2011). Using these cut-off points, most of urban and rural women were vitamin D deficient (43.9%) and sufficient (88.1%) respectively. 37.4% and 18.7% of urban subjects were vitamin D insufficient and sufficient respectively. Only 0.3% of rural subjects had vitamin D deficiency, while 11.6% of them had insufficient levels of vitamin D.

Urban women who were vitamin D deficient and rural women who were vitamin D insufficient had higher concentrations of serum i-PTH, whereas urban and rural women who were vitamin D sufficient had lower concentrations of serum i-PTH. In terms of vitamin D status, there was no significant difference in serum i-PTH levels between deficient vs insufficient (4.08 vs 3.90 pmol/L, p=0.665), deficient vs sufficient (4.08 vs 3.96 pmol/L, p=0.827) and insufficient vs sufficient (3.90 vs 3.96 pmol/L, p=0.727) levels of vitamin D in the overall population. The significant difference in PTH levels between vitamin D status only can be seen in rural women who were vitamin D insufficient and sufficient, with rural women with vitamin D insufficiency had significantly higher PTH levels compared to those rural women who were vitamin D sufficient (4.92 vs 3.96 pmol/L, p=0.003). Rural women had significantly higher levels

of serum i-PTH compared to urban women in the insufficient category of vitamin D status (4.92 vs 3.53 pmol/L, p=0.006) (Table 4.4).

Table 4.4: Classification of Vitamin D status and corresponding PTH levels

25 (OH)D[a]	Urban, n=107		Rural, n=293		Overall, n=400	
	n (%)	PTH level[b]	n (%)	PTH level[b]	n (%)	PTH level[b]
Deficient (<30 nmol/L)	47 (43.9)	4.07 (3.15-4.89)	1 (0.3)	4.33 (4.33-4.33)	48 (12.0)	4.08 (3.16-4.89)
Insufficient (30-<50 nmol/L)	40 (37.4)	3.53 (2.57-4.48)	34 (11.6)	4.92 (3.55-6.68)[c, d]	74 (18.5)	3.90 (3.09-5.39)
Sufficient (≥50 nmol/L)	20 (18.7)	3.39 (2.40-4.81)	258 (88.1)	3.96 (3.10-5.27)	278 (69.5)	3.96 (3.04-5.26)

[a] Vitamin D cut off points using IOM (Ross et al. 2011; IOM 2011).
[b] Data as median (Q25-Q75), PTH unit: pmol/L
[c]Significantly different from sufficient, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)
[d]Significantly different from urban

The normal range for serum i-PTH given by the manufacturer is 1.6-6.9 pmol/L (PTH, intact [package insert], 2010). Using these as cut-off points, higher percentages of women had been seen to have a normal (91.3%) value of serum i-PTH. On the other hand, only 0.5% of subjects had PTH levels below and 8.0% had PTH levels above the normal range of serum i-PTH. Only 6.3% of subjects with vitamin D deficiency had elevated PTH levels (data not shown).

No significant correlation was found between serum i-PTH and 25(OH)D levels (Spearman's rho=-0.048, p=0.342). The relationship between serum 25(OH)D and i-PTH levels in urban and rural women were further characterized in linear and non-linear models. The graphs can be seen in figure 4.1. Although some of the models fitted well, the predictive value of the models was low as it only explained by 2.2 – 5.0% of the variations.

Figure 4.1 (a) and (b) showed a linear relationship between serum 25(OH)D and PTH in urban and rural women. The equation as follows: a) urban: y = -0.011[25(OH)D] + 4.316; b) rural: y = -0.019[25(OH)D] + 5.752. The equation shows that a 1 unit increase in serum 25(OH)D levels in urban and rural women, decreased serum i-PTH levels by 0.001 (p=0.125) and 0.019 pmol/L (p=0.002), respectively. In rural, even though the linear relationship between serum 25(OH)D and i-PTH was statistically significant, but only 3.1% of the total variation of i-PTH can be explained by variation in 25(OH)D.

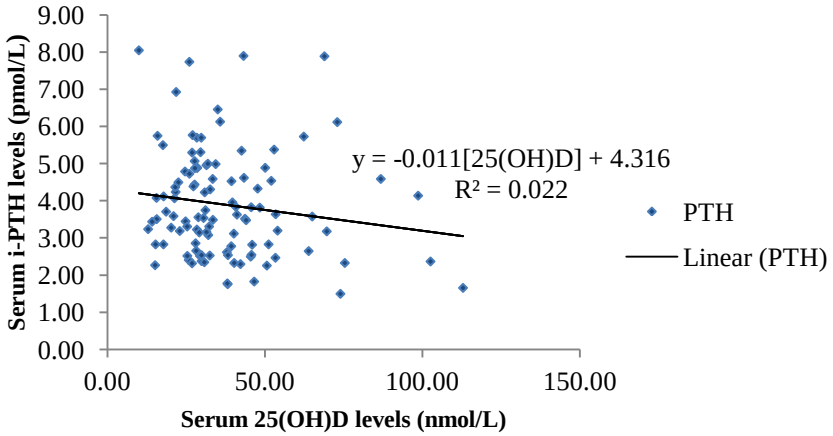
Figure 4.1 (c) and (d) illustrated a logarithmic relationship between serum 25(OH)D and PTH in urban and rural. The equation as follows: c) urban: y = -0.496ln[25(OH)D] + 5.640 (p=0.099); d) rural: y = -1.287ln[25(OH)D] + 9.858 (p=0.002). The equation indicates that an increase in 1 unit of serum 25(OH)D on the ln scale, decreased the serum i-PTH on the ln scale by 0.496 and 1.287, respectively. In rural, even though the relationship between ln[25(OH)D] and i-PTH was statistically significant, but only 3.1% of the total variation of i-PTH on the ln scale can be explained by variation in 25(OH)D on the ln scale.

Figure 4.1 (e) and (f) sketched a power relationship between serum 25(OH)D and PTH in urban and rural. The equation as follows: e) urban: $y = 6.331[25(OH)D]^{-0.156}$ (p=0.040); f) rural: $y = 13.541[25(OH)D]^{-0.284}$ (p=0.002). The p-value for this relationship is significant, however only 3.9% (urban) and 3.2% (rural) of the total variation of i-PTH on the power scale can be explained by variation in 25(OH)D on the power scale.

Figure 4.1 (g) and (h) drew an exponential relationship between serum 25(OH)D and PTH in urban and rural. The equation as follows: g) urban: $y = 4.221e^{-0.004x}$ (p=0.037); h) rural: $y = 5.4852e^{-0.004x}$ (p=0.002). For both urban and rural, the equation suggests that an increase in serum 25(OH)D level of 1 unit, decrease in serum i-PTH being multiplied by $e^{0.004}$. Even though the p-value in urban and rural was statistically significant, but only 4.1% (urban) and 3.2% (rural) of the total variation of i-PTH on the exponential scale can be explained by variation in 25(OH)D on the exponential scale.

Figure 4.1 (i) and (j) outlined a cubic relationship between serum 25(OH)D and PTH in urban and rural. The equation as follows: i) urban: $y = -2E\text{-}05[25(OH)D]^3 + 0.003[25(OH)D]^2 - 0.139[25(OH)D] + 6.027$ (p=0.148); j) rural: $y = -2E\text{-}05[25(OH)D]^3 + 0.003[25(OH)D]^2 - 0.139[25(OH)D] + 6.027$ (p=0.011). In rural, the positive value for the linear term imply that serum i-PTH increases initially with serum 25(OH)D. The negative value for the squared term indicates that past a certain spot, the level of serum i-PTH decreases. The positive value for the cubic term shows that serum i-PTH level rises again. In rural, although the p-value is significant, but only 3.8% of the total variation of i-PTH on the cubic scale can be explained by variation in 25(OH)D on the cubic scale.

a)



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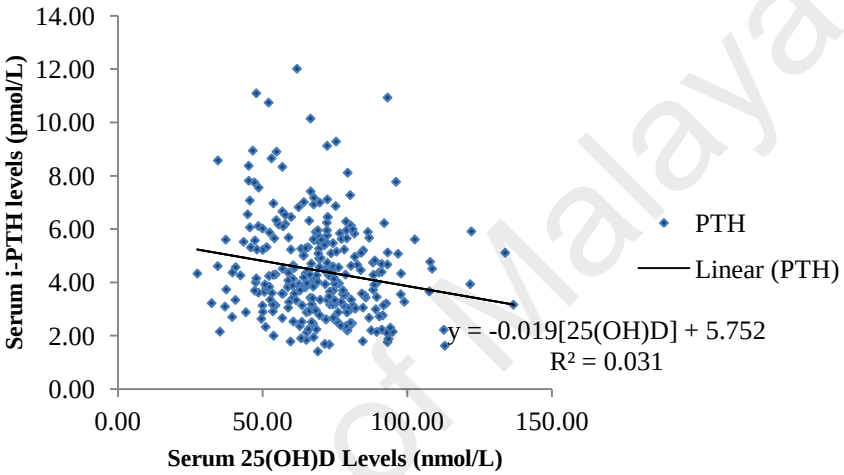


c)

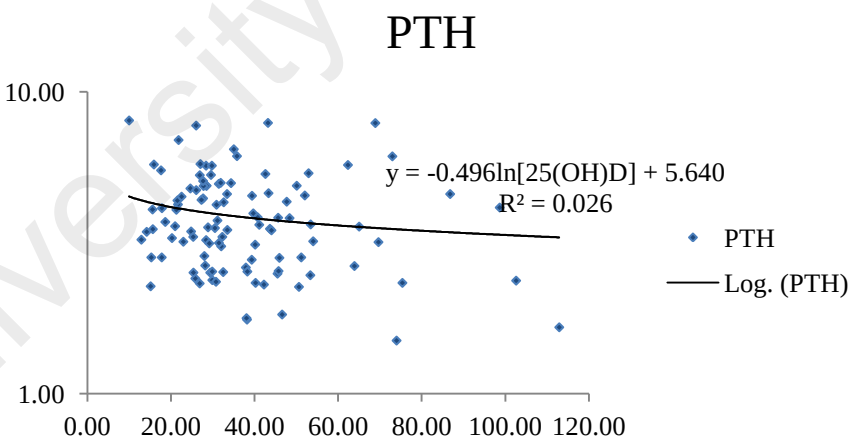
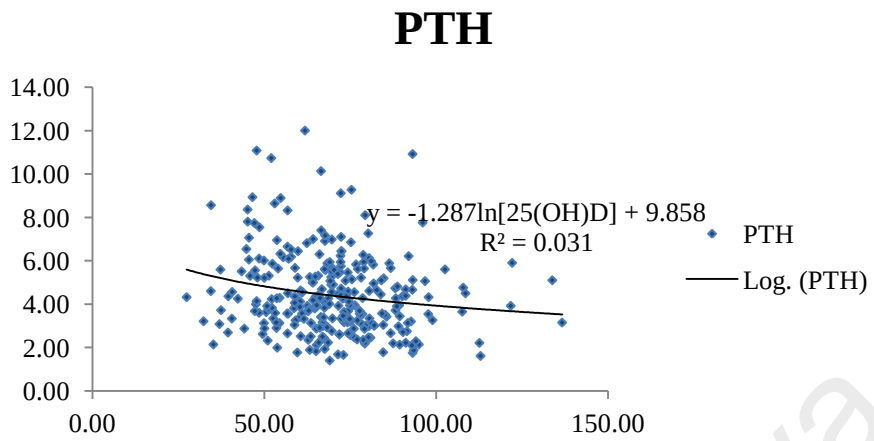
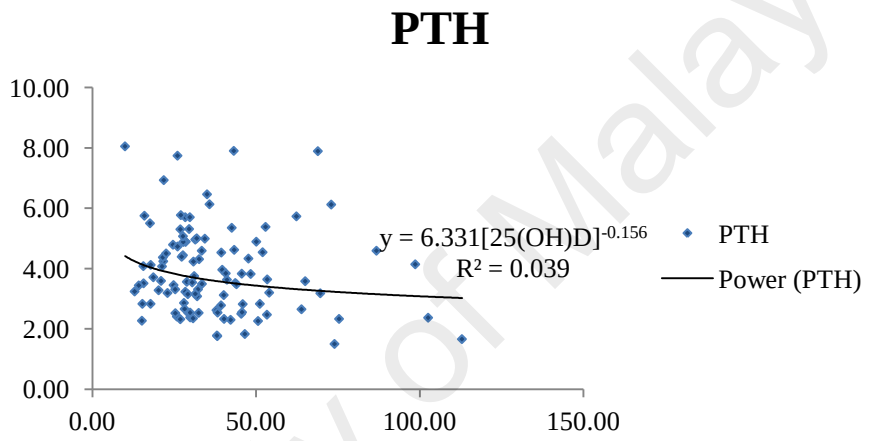


Figure 4.1, continued.

d)



e)



f)

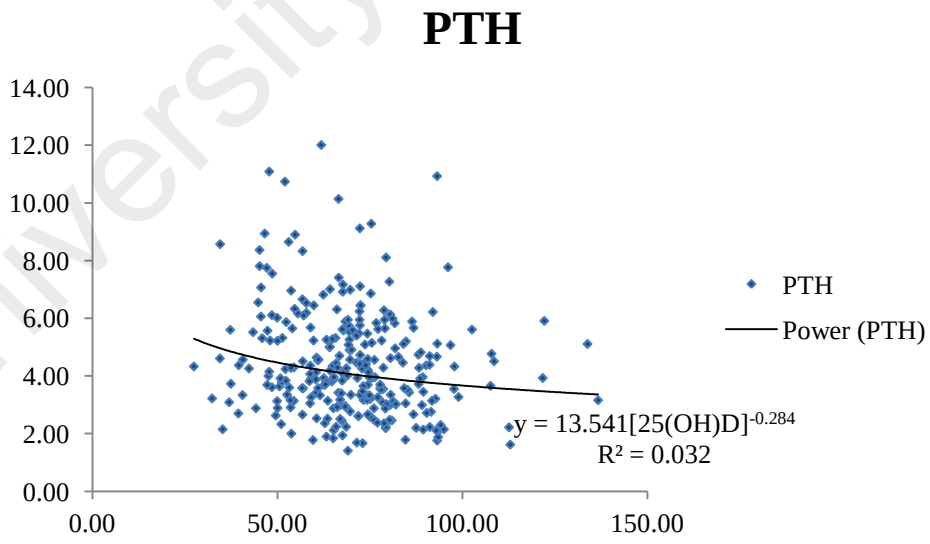
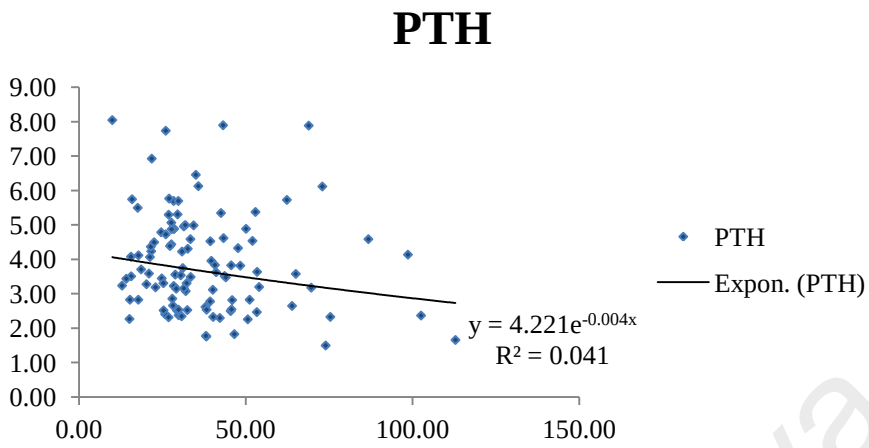
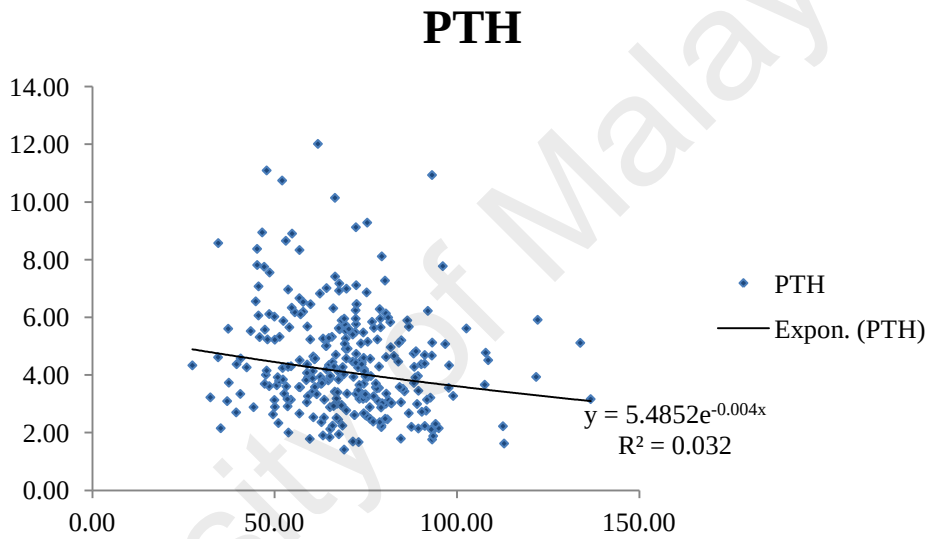


Figure 4.1, continued.

g)



h)



i)

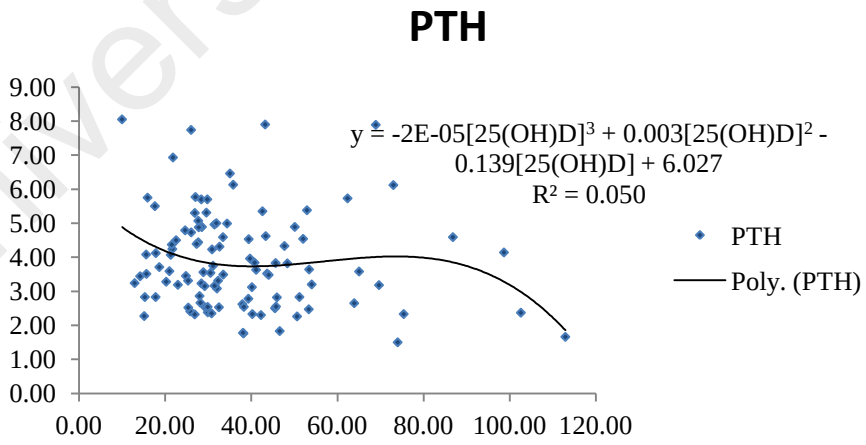


Figure 4.1, continued.

j)

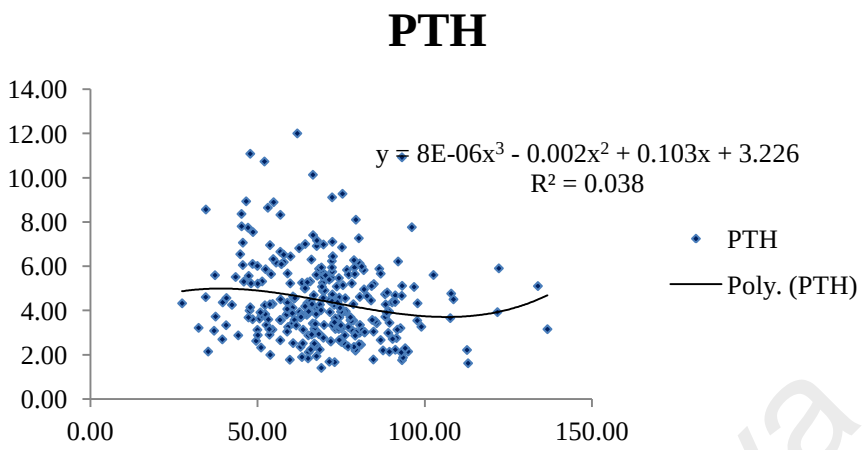


Figure 4.1: Linear and non-linear relationship between serum 25(OH)D and PTH in urban and rural subjects, a) Linear graph for urban b) Linear graph for rural c) Logarithmic graph for urban d) Logarithmic graph for rural e) Power graph for urban f) Power graph for rural g) Exponential graph for urban h) Exponential graph for rural i) Cubic graph for urban j) Cubic graph for rural.

4.4 Sun exposure

Table 4.5 features sun exposure. Rural women had significantly longer duration spent under the sun than urban women (7.83 hours/week vs 2.92 hours/week, p<0.001). Rural women also had a significantly higher sun index score than urban women although their fraction of BSA exposed to the sun was lower compared to urban women.

Table 4.5: Sun exposure

	Urban, n=107	Rural, n=293	p-value
Sun exposure per week (h)	2.92 (1.17-4.92)	7.83 (3.67-14.71)	<0.001
Fraction of BSA exposed to sunlight	0.21 (0.21-0.43)	0.12 (0.07-0.17)	<0.001
Sun Index[a]	0.72 (0.26-1.28)	0.89 (0.42-1.83)	0.018

Data as median (Q25-Q75)
[a] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight

Figure 4.2 illustrates the types of activities under the sun that were carried out by urban and rural women in a week. This includes occupation (e.g. farmers or rubber tappers),

transport (e.g. walking or cycling), activities at home (e.g. hanging clothes out to dry, sweeping the compound and gardening), home repair (e.g. washing or waxing cars and painting), exercise/sports (e.g. jogging and tai chi), and other activities (e.g. marketing and having feasts).

As shown in Figure 4.2, both urban (58.1%) and rural (51.3%) women spent more time under the sun while carrying out chores around the house (shown by a higher average of hours in a week). Rural women spent 5.74 hours/week for housework, while urban women spent 2.23 hours/week for the same activities. In rural women, 25.4% of their sun exposure comes from working outdoors, while only 1.3% of urban women sun exposure comes from their occupations (2.84 vs 0.05 hours/week). In urban women, doing other activities and transportation contributed to 15.9% and 13.0% of their sun exposure, with a mean of 0.61 and 0.50 hours/week respectively. Rural women also obtained 10.2% and 10.7% of their total sun exposure from doing other activities and transportation, with mean hours of sun exposure weekly of 1.14 and 1.20 respectively. A higher percentage of sun exposure in urban (10.7%) women comes from exercise, but only 2.2% of rural women experience sun exposure from exercise (0.41 vs 0.25 hours/week). Most urban and rural women spent less time on repairs at home; they only spent about 0.04 and 0.03 hours/week respectively on that activity.

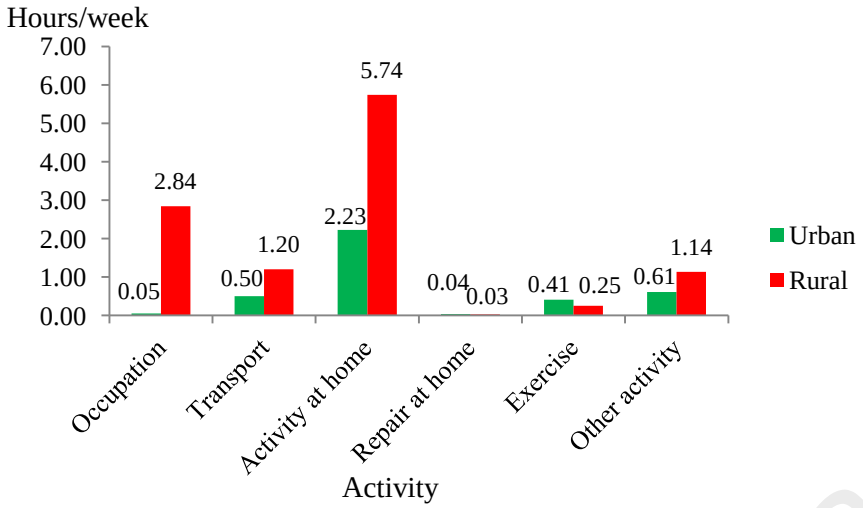


Figure 4.2: Type of activities (urban vs rural)

The majority of urban (74.8%) and rural (89.4%) women did not apply sunscreen when exposed to the sun. Only 6.5% of women living in urban areas reported frequent use and 18.7% occasional use of sunscreen. 5.5% of rural women said that they used sunscreen routinely and 5.1% admitted to using it occasionally (data not shown).

4.5 Vitamin D Food Intake

Rural women had higher vitamin D intake than urban women (5.23 vs 4.61 µg/day) respectively.

In terms of vitamin D, rural women had significantly higher intake of fish, oily fish and shellfish compared to urban women. However, there were no significant difference between urban and rural women in the consumption of other types of vitamin D food (i.e. milk and milk products, soy, beverages, cereals products and meat and meat products) (Table 4.6).

Table 4.6: Vitamin D Food Intake

	Urban, n=107	Rural, n=293	p-value
Milk and products, µg/day	1.05 (0.30-2.66)	1.09 (0.35-2.58)	0.450
Soy, µg/day	0 (0-0)	0 (0-0)	0.555
Beverages, µg/day	0 (0-0)	0 (0-0)	0.052
Cereals and products, µg/day	1.16 (0.45-0.93)	1.01 (0.46-1.58)	0.355
Fish, oily fish and shellfish, µg/day	1.05 (0.09-2.83)	1.81 (0.85-3.56)	<0.001
Meat and products, µg/day	0.21 (0.09-0.29)	0.19 (0.06-0.31)	0.169
Total vitamin D intake, µg/day	4.61 (2.66-7.41)	5.23 (3.31-8.45)	0.050

Data as median (Q25-Q75)

The highest percentage of vitamin D food source among urban women was milk and milk products (30.7%). Other vitamin D food sources consumed by urban women were fish/oily fish and/or shellfish (30.1%), cereal products (29.4%), meat and meat products (7.1%), beverages (1.9%), and soy (0.9%) (Figure 4.3).

In contrast, rural women have a higher intake of fish/oily fish and/or shellfish (41.0%). This was followed by milk and milk products (27.4%), cereal and its products (22.5%), meat and its products (5.7%), beverages (2.3%) and soy (1.1%) (Figure 4.4)

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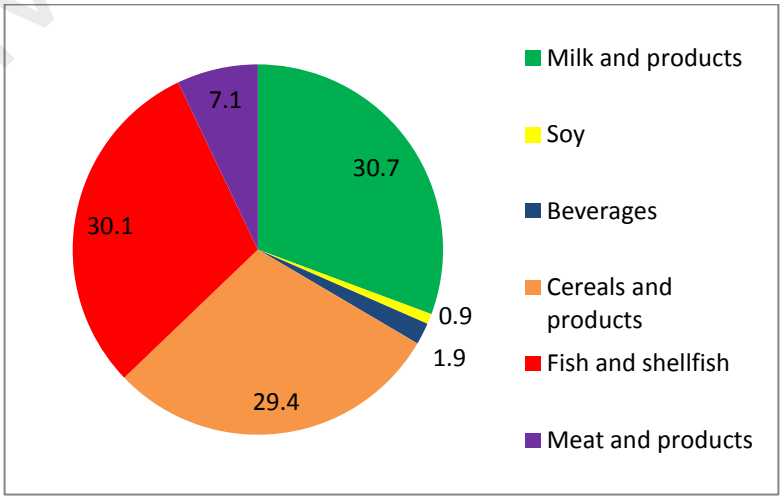

Figure 4.3: Vitamin D Food Intake in Urban Women

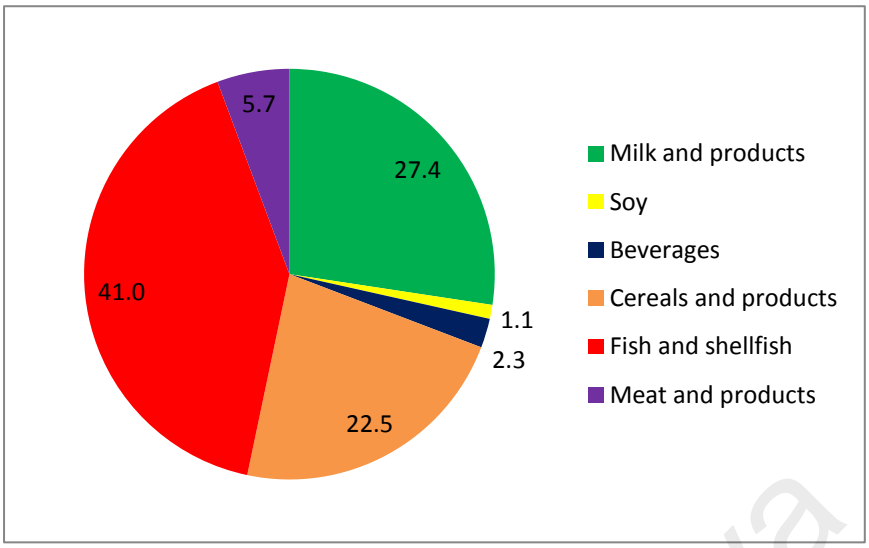

Figure 4.4: Vitamin D Food Intake in Rural Women

The RNI for vitamin D for Malaysian women aged 30-50 years, 51-65 years and >65 years is 5, 10 and 15 µg/day (200, 400, 600 IU/day) respectively (RNI Malaysia, 2005). Only 7.5% and 19.1% of urban and rural women met the RNI for vitamin D for Malaysian women of this age group (Table 4.7).

Table 4.7: Recommended Nutrient Intakes (RNI) for Vitamin D

	Urban, n=107	Rural, n=293	p-value
Meet RNI	8 (7.5)	56 (19.1)	0.05
Did not meet RNI	99 (92.5)	237 (80.9)	

Data as n (%)

4.6 Calcium Food Intake

Rural women had significantly higher median intake of calcium compared to urban women [532 (330-737) vs 423 (324-562) mg/day, p=0.001] respectively. In terms of calcium food, urban women had significantly higher consumption of vegetables, fruits, and cereals and its products. On the other hand, rural women had significantly higher intake of fish and shellfish and western fast food/kuehs. However, no significant

difference was seen between urban and rural women regarding the intake of milk and milk products, meat and its products, legumes, tubers, mixed dishes and beverages (Table 4.8).

Table 4.8: Calcium food intake

	Urban, n=107	Rural, n=293	p-value
Milk and products, mg/day	114.33 (52.50-226.93)	112.70 (29.67-240.48)	0.998
Vegetables, mg/day	73.64 (46.21-98.04)	51.09 (30.43-85.58)	0.002
Fruits, mg/day	15.22 (7.92-25.49)	9.33 (3.18-22.52)	0.001
Meat and products, mg/day	20.57 (11.65-32.82)	18.59 (9.63-33.93)	0.618
Fish and shellfish, mg/day	29.01 (14.59-64.48)	117.44 (51.35-187.59)	<0.001
Cereals and products, mg/day	25.33 (10.14-43.24)	19.12 (9.17-36.51)	0.024
Legumes, mg/day	28.88 (16.25-61.06)	24.67 (10.83-48.21)	0.087
Tubers, mg/day	1.86 (0.91-4.72)	2.54 (1.07-5.18)	0.051
Mixed dishes, mg/day	20.72 (11.98-32.17)	19.56 (7.34-36.29)	0.338
Western fast food, mg/day	1.64 (0-4.56)	3.76 (1.03-9.86)	<0.001
Beverages, mg/day	12.98 (6.04-32.61)	15.19 (6.01-35.41)	0.604
Total calcium intake, mg/day	423.49 (324.28-561.67)	529.84 (328.17-736.09)	0.001

Data as median (Q25-Q75)

The highest percentage of calcium food intake for urban women was milk and milk products (30.1%). Other calcium sources consumed by urban women were vegetables (18.4%), fish and shellfish (10.4%), legumes (9.8%), cereals and its products (7.2%), fruits (6.1%), meat and meats products (6.0%), beverages (5.6%), fruits (4.9%), tubers (0.8%) and western fast food (0.7) (Figure 4.5). Rural women also had a higher intake of milk and milk products (26.8%). This was followed by fish and shellfish (24.8%), vegetables (14.1%), legumes (7.5%), beverages (5.7%), mixed dishes (5.4%), cereals and its products (5.2%), meat and meat products (5.2%), fruits (3.2%), western fast food (1.4%) and tubers (0.9%) (Figure 4.6).

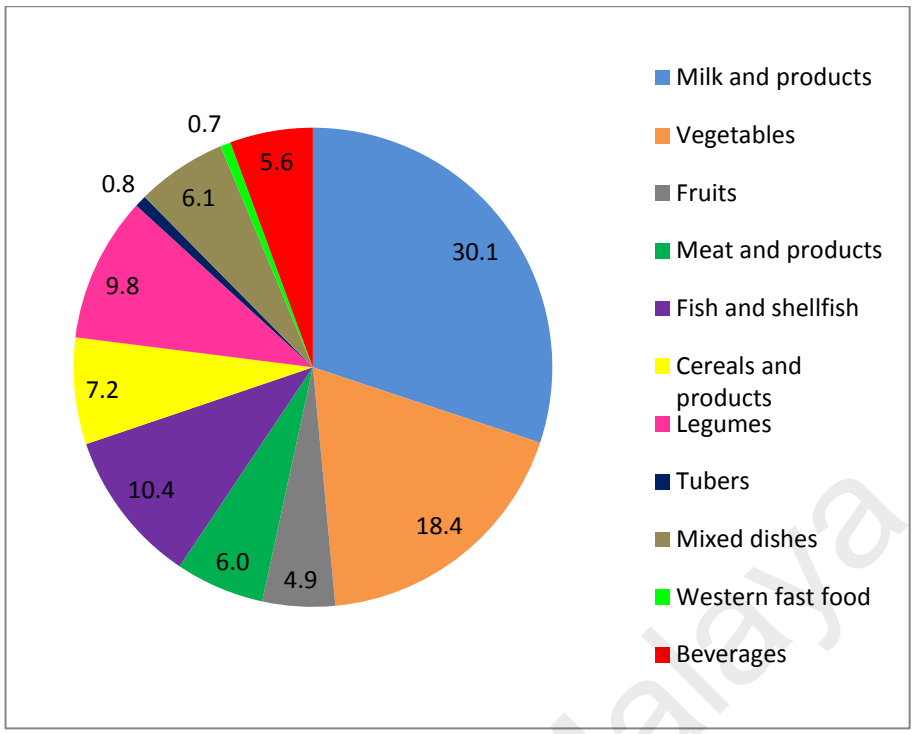

Figure 4.5: Calcium Food Intake in Urban Women

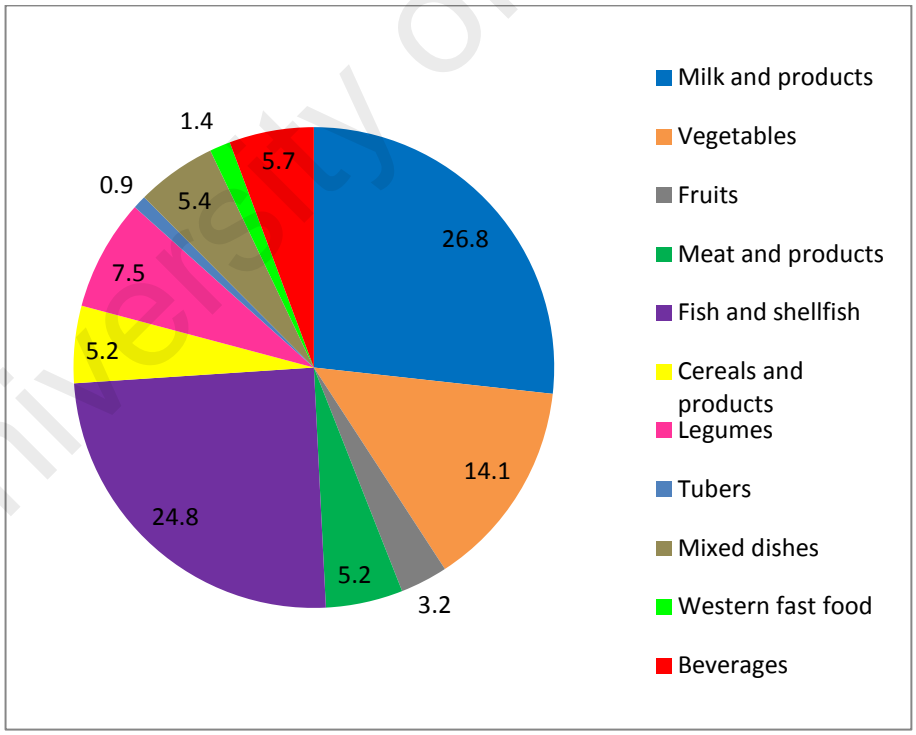

Figure 4.6: Calcium Food Intake in Rural Women

According to RNI Malaysia (2005), the recommended calcium intake for Malaysian women aged 30-50 years and >50 years is 800 and 1000 mg/day respectively. 8.9% of rural women met the RNI for calcium compared to 0.9% of urban women (Table 4.9).

Table 4.9: RNI for Calcium

	Urban, n=107	Rural, n=293	p-value
Meet RNI	1 (0.9)	26 (8.9)	0.05
Did not meet RNI	106 (99.1)	267 (91.1)	

Data as n (%)

4.7 Energy and other nutrient intake

Table 4.10 shows nutrient intake of urban and rural women per day. Urban women had a significantly higher intake of calories (1380 vs 1139 kcal/day, p<0.001), protein (56 vs 32 g/day, p<0.001), carbohydrate (188 vs 170 g/day, p=0.003) and fat (42 vs 29 g/day, p<0.001) compared to rural women. On the other hand, rural women had a higher intake of vitamin D compared to urban women (3.03 vs 2.49 µg/day, p=0.353).

Table 4.10: Nutrient Intake per Day

	Urban, n=107	Rural, n=293	p-value
Calories, kcal	1380 (1119-1667)	1139 (844-1512	<0.001
Protein, g	56 (43-75)	32 (66)	<0.001
Carbohydrate, g	188 (158-235)	170 (122-223)	0.003
Fat, g	42 (21-61)	29 (17-40)	<0.001
Vitamin D, µg/day	2.49 (0.75-5.35)	3.03 (0.81-6.22)	0.353

Data as median (Q25-Q75)

4.8 Supplements

Table 4.11 illustrates the supplement intake of the subjects. Supplements were categorized into cod liver oil, vitamin D (e.g. calcitriol), calcium (e.g. calcium carbonate, calcium lactate), vitamin D plus calcium (e.g. Eurobio® BioCal D 600, Bio-Enhanced Calcium Plus) and multivitamins (e.g. Pharmaton, Blackmore Multi Vitamin, Flavettes Daily Plus). The percentage of urban women who were on cod liver oil (13.1 vs 2.0%), vitamin D (5.6 vs 0.7%), calcium (46.7 vs 3.4%), vitamin D plus calcium (3.7 vs 0.3%) and multivitamin (19.6 vs 1.0%) were significantly higher compared to rural women.

Table 4.11: Supplements

	Urban, n=107	Rural, n=293	p-value
Cod liver oil			<0.001
Yes	14 (13.1)	6 (2.0)	
No	93 (86.9)	287 (98.0)	
Vitamin D			0.002
Yes	6 (5.6)	2 (0.7)	
No	101 (94.4)	291 (99.3)	
Calcium			<0.001
Yes	50 (46.7)	10 (3.4)	
No	57 (53.3)	283 (96.6)	
Vitamin D+Calcium			0.007
Yes	4 (3.7)	1 (0.3)	
No	103 (96.3)	292 (99.7)	
Multivitamin			<0.001
Yes	21 (19.6)	3 (1.0)	
No	86 (80.4)	290 (99.0)	

Data as n (%)

Vitamin D containing supplements include cod liver oil, vitamin D, vitamin D plus calcium, and multivitamins. There were higher percentages of urban women who were on vitamin D containing supplements compared to rural women (32.7 vs 4.1%) (Table 4.12).

Table 4.12: Vitamin D containing supplement

	Urban, n=107	Rural, n=293	P-value
Take vitamin D containing supplement	35 (32.7)	12 (4.1)	<0.001
Did not take vitamin D containing supplement	72 (67.3)	281 (95.9)	

Data as n (%)

4.9 Dietary Vitamin D Intake: Comparison between FFQ and 24 Hours Dietary Recalls

4.9.1 Intake of Vitamin D from FFQ and 24 Hour Dietary Recall

Table 4.13 presents the median dietary intake of vitamin D from FFQ and 24 hour dietary recall. The median vitamin D intake from FFQ was significantly higher compared to the 24 hour dietary recall.

Table 4.13 : Median dietary intake of vitamin D from FFQ and 24 hours dietary recalls

	FFQ	24 Hours Dietary Recalls	p-value
Vitamin D Intake, µg/day	5.02 (2.98-8.21)	2.82 (0.76-6.19)	<0.001

Data as median (Q25-Q75)

Figure 4.7 displays a scatter plot showing the relationship between vitamin D intake derived from FFQ and a 24 hour dietary recall. Vitamin D intake from FFQ correlated positively with vitamin D intake from the 24 hour dietary recall (Spearman's rho=0.341, p<0.001).

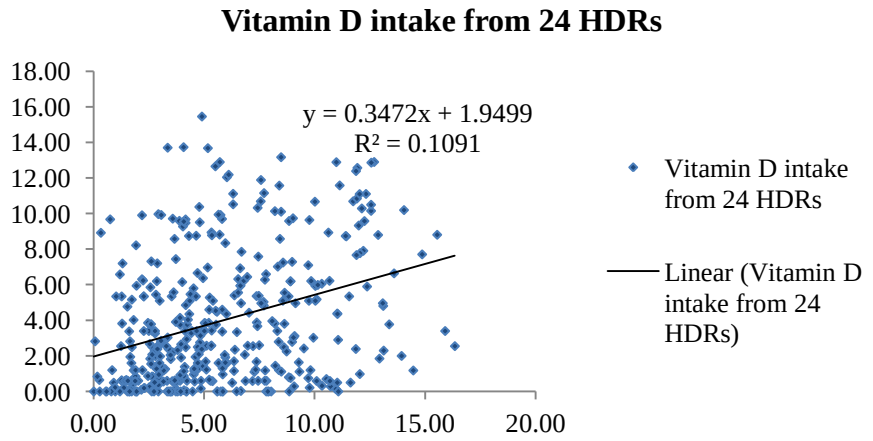

Figure 4.7 : Linear relationship between vitamin D intake derived from FFQ and 24 HDRs

4.9.2 Joint Classification of Vitamin D Intake Assessed by the FFQ and 24 Hour Dietary Recall

Cross classification analysis was performed in order to determine whether vitamin D intake from FFQ and 24 hour dietary recall have a good agreement or have been misclassified. Vitamin D intake from FFQ and the 24 hour dietary recall were divided into quartiles. The cross-classification analysis showed that 295 subjects (73.9%) had been correctly classified (within one quartile) (Magkos, et al. 2006), while 25 subjects (6.3%) were grossly misclassified (the lowest quartile for one method and the highest quartile for the other) (Magkos, et al. 2006) FFQ and 24 hours dietary recalls (Table 4.14).

Table 4.14 : Joint classification of Vitamin D Intake Assessed by the FFQ and 24 Hour Dietary Recall

	24 hours dietary recalls quartiles				
FFQ quartiles	1	2	3	4	Total
1	41 (41.4)	26 (26.0)	21 (21.0)	12 (12.0)	100
2	25 (25.3)	31 (31.0)	26 (26.0)	18 (18.0)	100
3	20 (20.2)	23 (23.0)	29 (29.0)	28 (28.0)	100
4	13 (13.1)	20 (20.0)	24 (24.0)	42 (42.0)	99
Total	99	100	100	100	399

Table 4.14, continued.

Data as n (%)
Quartiles 1 to 4 for FFQ: 0.01-2.98, 2.98-5.01, 5.02-8.20, 8.22-16.34 µg/day (0.4-119.2, 119.2-200.4, 200.8-328, 328.8-653.6 IU/day)
Quartiles 1 to 4 for 24 hours recalls: 0-0.75, 0.76-2.79, 2.82-6.15, 6.19-15.46 µg/day (0-30, 30.4-111.6, 112.8-246, 247.6-618.4 IU/day)

4.9.3 Specificity and Sensitivity of the Questionnaire

Specificity is described as 'the proportion of women having dietary intake measured by FFQ and 24 hour dietary recall below the RNI for vitamin D'. Sensitivity is defined as 'the proportion of women with a dietary vitamin D intake above RNI as calculated by FFQ and 24 hour dietary recall' (Chee, et al. 2002). The specificity of the questionnaire was 86.7% since 307 out of 354 subjects did not meet RNI for vitamin D. The sensitivity of the questionnaire was 44.4%, where 20 out of 45 women met RNI for vitamin D (Table 4.15).

Table 4.15: RNI for vitamin D assessed by FFQ and 24 hours dietary recalls

	24 Hours Dietary Recalls	
FFQ	Did not meet RNI, n=354	Meet RNI, n=45
Meet RNI[a]	47 (13.3)	20 (44.4)
Did not meet RNI	307 (86.7)	25 (55.6)

Data as n (%)
a The RNI for vitamin D intake for Malaysian women aged 30-50 years, 51-65 years and >65 years is 5, 10 and 15 µg/day (200, 400, 600 IU/day) respectively (Ismail, et al. 2005)

4.9.4 Bland-Altman Plots

The mean difference between vitamin D intake from the FFQ and 24 hour dietary recall was +1.82 µg/day (+72.8 IU/day). The 95% limit of agreement were -6.45 to +10.09 µg/day (-258 to + 403.6 IU/day) (Figure 4.8).

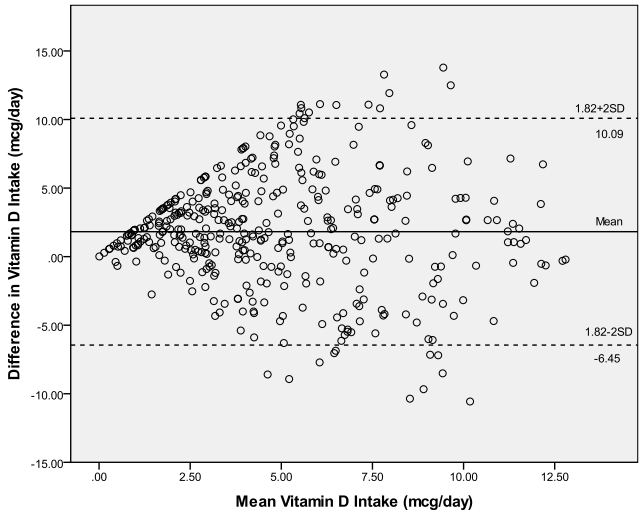

Figure 4.8: Bland-Altman plots to assess agreement and systematic difference between the FFQ and 24 hours dietary recalls for vitamin D intake. ‘‘Vitamin D difference’’ (y-axis) is the difference in vitamin D intake by the FFQ minus that by the 24-h recall, while ‘‘vitamin D mean’’ (x-axis) is the mean of vitamin D intake by the two methods. The mean (solid line) and the 95% CI (broken lines) of the difference were shown (Magkos, et al. 2006).

4.10 Serum 25(OH)D levels, dietary intake and sun exposure according to age groups

Figure 4.9 illustrates the median 25(OH)D levels according to age groups in urban and rural women. Overall, vitamin D levels decrease across the age groups. Women aged <50 years (75.10 nmol/L) had significantly higher 25(OH)D levels than those women aged 50-59 (66.80 nmol/L), 60-69 (53.43 nmol/L) and ≥70 (35.80 nmol/L) years respectively. Rural women aged 50-59 (69.04 vs 29.58 nmol/L, p<0.001), 60-69 (68.65 vs 34.38 nmol/L, p<0.001) and ≥70 (73.83 vs 27.70 nmol/L, p=0.001) years had significantly higher vitamin D levels compared to those urban women of the same age.

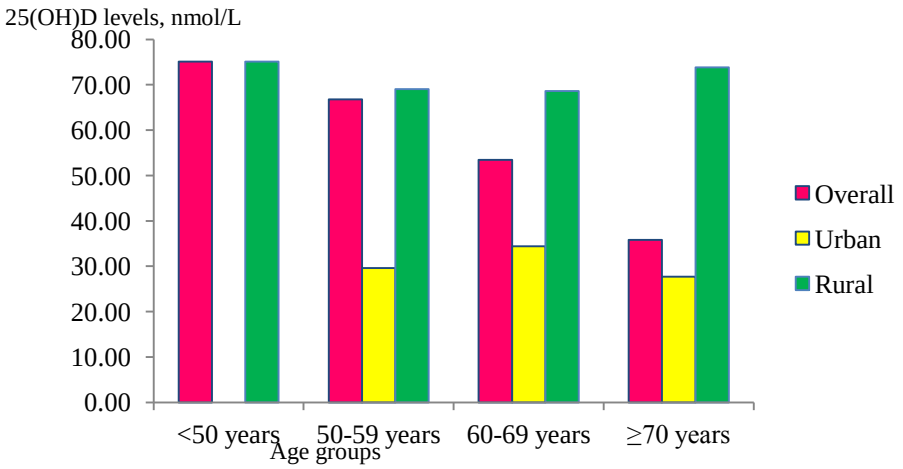

Figure 4.9 : Median 25(OH)D levels according to age groups in urban and rural women

Table 4.16 shows dietary vitamin D and calcium intake among age groups in urban and rural women. There were no significant differences in the vitamin D and calcium intake between each age group. Overall, women aged 60-69 years have a higher consumption of dietary vitamin D (5.51 µg/day) than other age groups. On the other hand, women aged <50 years have higher consumption of dietary calcium (590 mg/day) compared to women in other age groups. According to area, only rural women aged 60-69 years had significantly higher consumption of dietary vitamin D than urban women aged 60-69 years (5.88 vs 4.61 µg/day, p=0.045). Rural women aged 50-59 years had higher calcium intake compared to urban women in the same age group (528 vs 397 mg/day, p=0.038).

Table 4.16 : Dietary intake among age groups in urban and rural women

	<50 years			50-59 years			60-69 years			≥70 years		
	Total, n=23	Urban, n=0	Rural, n=23	Total, n=243	Urban, n=43	Rural, n=200	Total, n=119	Urban, n=55	Rural, n=64	Total, n=15	Urban, n=9	Rural, n=6
Total vitamin D intake, µg/day	4.57 (2.61-10.03)	NA	4.57 (2.61-10.03)	4.89 (2.98-8.31)	4.04 (2.46-7.69)	5.08 (3.30-8.38)	5.51 (2.95-7.82)	4.61 (2.78-7.27)	5.88 (3.81-8.81)[a]	4.90 (3.49-10.32)	5.82 (4.01-10.27)	4.29 (2.91-10.88)
Calcium intake, mg/day	590 (420-894)	NA	590 (420-894)	504 (324-727)	397 (305-619)	528 (327-742)[a]	445.51 (318-596)	434 (324-524)	500 (300-730)	543 (362-713)	543 (386-760)	543 (325-752)

Data as median (Q25-Q75)

[a] Significant difference between groups at $p<0.05$

Table 4.17 illustrates sun exposure according to age. Overall, women aged less than 50 years (11.33 hours/week) had a significantly higher duration of sun exposure per week than women aged 50-59 years (7.00 hours/week), 60-69 years (4.17 hours/week) and ≥70 years (2.33 hours/week) respectively. Conversely, women aged ≥70 years (21%) had a significantly higher fraction of BSA exposure to the sun than women aged <50 years (9%) and 50-59 years (13%) respectively. However, there were no significant differences in sun index among age groups. Rural women aged 50-59 (8.03 vs 2.83 hours/week, $p<0.001$) and 60-69 (5.54 vs 3.08 hours/week, $p<0.001$) years spent significantly longer time under the sun than urban women in the same age. On the other hand, a lower fraction of BSA exposure to the sun was seen in rural women aged 50-59 (12 vs 21%, $p<0.001$), 60-69 (14 vs 21%, $p<0.001$) and ≥70 (11 vs 28%, $p=0.001$) years compared to urban women in the same age group. Only rural women aged 50-59 years had a significantly higher sun index score than urban women aged 50-59 years (0.94 vs 0.68, $p=0.017$).

Table 4.17 : Sun exposure among age groups in urban and rural women

	<50 years			50-59 years			60-69 years			≥70 years		
	Total, n=23	Urban, n=0	Rural, n=23	Total, n=243	Urban, n=43	Rural, n=200	Total, n=119	Urban, n=55	Rural, n=64	Total, n=15	Urban, n=9	Rural, n=6
Hours of sun exposure per week	11.33 (4.75-24.50)[b,c,d]	NA	11.33 (4.75-24.50)	7.00 (3.25-14.00)[e,f]	2.83 (1.17-4.50)	8.03 (4.04-16.09)[h]	4.17 (2.25-9.25)	3.08 (1.25-5.50)	5.54 (3.00-12.75)[h]	2.33 (1.67-7.58)	2.58 (1.54-5.79)	2.25 (1.55-11.38)
Fraction of BSA exposed to sunlight	0.09 (0.07-0.17)[c,d]	NA	0.09 (0.07-0.17)	0.13 (0.07-0.20)[e,f]	0.21 (0.19-0.41)	0.12 (0.07-0.17)[h]	0.17 (0.07-0.21)	0.21 (0.18-0.43)	0.14 (0.07-0.18)[h]	0.21 (0.15-0.33)	0.28 (0.21-0.45)	0.11 (0.07-0.17)[g]
Sun Index[a]	1.15 (0.59-1.97)	NA	1.15 (0.59-1.97)	0.88 (0.42-1.81)	0.68 (0.26-1.28)	0.94 (0.48-1.92)[g]	0.72 (0.31-1.44)	0.72 (0.23-1.31)	0.63 (0.34-1.47)	0.90 (0.23-1.11)	1.01 (0.40-1.61)	0.27 (0.15-1.18)

Data as median (Q25-Q75)

[a] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight

[b] Significantly different from 50-59 years, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[c] Significantly different from 60-69 years, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[d] Significantly different from ≥70 years, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[e] Significantly different from 60-69 years, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[f] Significantly different from ≥70 years, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[g] Significant difference between groups at $p<0.05$

[h] Significant difference between groups at $p<0.001$

4.11 Serum 25(OH)D and PTH concentrations, skin colour, dietary intake and sun exposure according to ethnicity

Table 4.18 illustrates serum 25(OH)D and PTH levels and skin colour among three ethnic groups in urban and rural women. All ethnic groups in the rural area had a significantly higher concentrations of vitamin D compared to urbanites. Overall, Malays (68.38 nmol/L) had significantly higher vitamin D levels compared to Chinese (41.13 nmol/L) and Indian (49.63 nmol/L) women respectively. Malays also have a significantly darker skin colour than the Chinese. On the other hand, Malay and Chinese had significantly lighter skin colour compared to Indian women. However, there were no significant differences in PTH levels among the different ethnicities.

Table 4.19 shows dietary vitamin D and calcium intake among three ethnic groups in urban and rural women. Overall, Malays (5.54 µg/day) had significantly higher dietary vitamin D intake compared to Chinese (3.58 µg/day) and Indian (4.64 µg/day) respectively. Malay (547 mg/day) women also had a significantly higher consumption of dietary calcium than Chinese (395 mg/day) and Indian (385 mg/day) women respectively.

Table 4.18 : 25(OH)D and PTH levels and skin colour among three ethnic groups in urban and rural

Variables	Malay			Chinese			Indian		
	Overall, n=267	Urban, n=21	Rural, n=246	Overall, n=67	Urban, n=56	Rural, n=11	Overall, n=66	Urban, n=30	Rural, n=36
25(OH)D levels, nmol/L	68.38 (56.80-78.83)[b, c]	27.05 (19.83-36.50)[b]	69.60 (60.28-79.35)[c, f]	41.13 (28.50-66.10)	36.23 (27.95-49.68)	78.65 (66.10-92.73)[d,f]	49.63 (30.82-66.93)	29.90 (25.98-43.53)	63.23 (50.90-72.25)[f]
PTH, pmol/L	3.98 (3.14-5.32)	3.59 (3.12-5.63)	4.02 (3.13-5.31)	3.63 (3.12-4.77)	3.61 (2.83-4.59)	4.29 (3.14-5.52)	4.08 (2.64-5.50)	3.56 (2.51-4.86)	4.30 (3.21-6.23)
Darkest skin colour score	12 (11-13)[b, c]	11 (9-12)	12 (11-13)[e]	8 (6-9)[d]	7 (6-9)	10 (6-12)	15 (13-16)	13 (12-16)	15 (14-16)[e]
Lightest skin colour score	5 (4-8)[b, c]	6 (4-8)	5 (4-8)	2 (1-4)[d]	2 (1-3)	5 (3-5)[f]	11 (9-12)	9 (8-11)	12 (10-14)[f]
Delta skin colour[a]	6 (3-7)[c]	5 (3-6)	6 (3-8)	5 (4-7)[d]	5 (4-7)	3 (3-7)	4 (3-5)	4 (3-5)	3 (2-4)[e]

Data as median (Q25-Q75)

[a] Delta skin colour/the changes of the skin colour = Darkest skin colour – Lightest skin colour

[b] Significantly different from Chinese,p<0.0167 (Mann-Whitney U tests with Bonferroni correction)

[c] Significantly different from Indian, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)

[d] Significantly different from Indian, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)

[e] Significant difference between groups at p<0.05

[f] Significant difference between groups at p <0.001

Table 4.19 : Dietary vitamin D and calcium intake among three ethnic groups in urban and rural

Variables	Malay			Chinese			Indian		
	Overall, n=267	Urban, n=21	Rural, n=246	Overall, n=67	Urban, n=56	Rural, n=11	Overall, n=66	Urban, n=30	Rural, n=36
Dietary vitamin D intake, µg/day	5.54 (3.39-8.90)[a, b]	6.01 (4.70-10.67)	5.51 (3.31-8.83)	3.58 (2.24-7.27)	3.48 (2.47-7.12)	4.16 (1.73-7.37)	4.64 (2.93-6.43)	4.85 (2.76-6.74)	4.56 (3.37-6.29)
Dietary calcium intake, mg/day	547 (371-767)[a, b]	472 (377-642)	555 (367-773)	395 (264-524)	388 (277-505)	518 (233-589)	385 (283-583)	430 (360-587)	337 (227-569)[c]

Data as median (Q25-Q75)

[a] Significantly different from Chinese, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)

[b] Significantly different from Indian, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)

[c] Significant difference between groups at p<0.05

Table 4.20 shows sun exposure among three ethnic groups in urban and rural women. Overall, Malay (7.83 hours/week) had significantly higher hours of sun exposure/week compared to Chinese (3.33 hours/week) and Indian (3.50 hours/week) women respectively. Chinese (26%) and Indian (21%) women also had significantly higher fraction of BSA exposure to the sun than Malay (10%) women. However, there were no significant differences in the sun index between the three races. All ethnicities from the rural area had significantly higher duration of sun exposure/week compared to their urban counterparts (Malay: 8.03 vs 3.67 hours/week, p=0.001; Chinese: 6.92 vs 2.56 hours/week, p=0.002; Indian: 4.54 vs 3.04 hours/week, p=0.020). Chinese and Indian from urban areas also had a significantly higher fraction of BSA exposure to the sun compared to Chinese (33 vs 20%, p=0.002) and Indian (28 vs 21%, p<0.001) women from rural areas. Only Malay women from rural areas had significantly higher sun exposure index score compared to the urban Malays (0.88 vs 0.40, p=0.003).

Table 4.20 : Sun exposure among three ethnic groups in urban and rural

Variables	Malay			Chinese			Indian		
	Overall, n=267	Urban, n=21	Rural, n=246	Overall, n=67	Urban, n=56	Rural, n=11	Overall, n=66	Urban, n=30	Rural, n=36
Hours of sun exposure per week	7.83 (3.67-15.00)[b, c]	3.67 (1.59-6.84)	8.03 (3.96-16.27)[e]	3.33 (1.25-6.67)	2.56 (1.17-4.73)	6.92 (5.00-25.42)[e]	3.50 (1.54-7.00)	3.04 (1.32-4.29)	4.54 (1.94-9.83)[e]
Fraction of BSA exposed	0.10 (0.07-0.17)[b, c]	0.07 (0.07-0.19)	0.10 (0.07-0.17)	0.26 (0.21-0.45)[d]	0.33 (0.21-0.45)	0.20 (0.09-0.21)[e]	0.21 (0.18-0.23)	0.28 (0.21-0.40)	0.21 (0.17-0.21)[f]
Sun index[a]	0.84 (0.40-1.73)	0.40 (0.13-0.94)	0.88 (0.42-1.79)[e]	0.83 (0.35-1.89)	0.71 (0.26-1.75)	1.50 (0.74-2.68)	0.97 (0.41-1.50)	1.01 (0.45-1.24)	0.86 (0.33-1.79)

Data as median (Q25-Q75)

[a] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight

[b] Significantly different from Chinese, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[c] Significantly different from Indian, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[d] Significantly different from Indian, $p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[e] Significant difference between groups at $p<0.05$

[f] Significant difference between groups at $p<0.001$

4.12 Vitamin D Status and Other Parameters

Table 4.21 shows age, skin colour and 25(OH)D concentrations in both the urban and rural population according to vitamin D status. The rural population had significantly higher 25(OH)D concentrations compared to urban women in the vitamin D insufficient (45.40 vs 38.80 nmol/L, $p<0.001$) and sufficient (72.24 vs 64.46 nmol/L, $p=0.048$) groups. On the other hand, rural women registered insufficient (56 vs 62 years, $p<0.001$) and sufficient (56 vs 64 years, $p<0.001$) were significantly younger than urban women in those groups respectively. In terms of skin colour, those women who had vitamin D sufficient have darker skin colour under the arms (6 vs 4, $p=0.016$) and on the back of their hands (12 vs 11, $p<0.001$) compared to those deficient in vitamin D. According to area, rural women who were vitamin D insufficient (6 vs 4, $p=0.001$) and those who had sufficient vitamin D (6 vs 2, $p<0.001$) had darker under-arm skin colour than urban women. Vitamin D insufficient rural women (13 vs 9, $p=0.001$) and those with sufficient vitamin D (12 vs 9, $p<0.001$) also had darker skin colour on the back of their hands compared to their urban counterparts.

Table 4.21 : Age, skin colour and 25(OH)D concentrations among urban and rural women according to vitamin D status

	Vitamin D deficiency (<30 nmol/L)			Vitamin D insufficiency (30-50 nmol/L)			Vitamin D sufficiency (≥50 nmol/L)		
Parameters	Overall, n=48	Urban, n=47	Rural, n=1	Overall, n=74	Urban, n=40	Rural, n=34	Overall, n=278	Urban, n=20	Rural, n=258
25(OH)D concentrations, nmol/L[a]	25.60 (19.05-28.15)[c,d]	25.38 (18.65-28.20)	27.45 (27.45-27.45)	40.95 (35.18-45.91)[e]	38.80 (32.53-43.32)	45.40 (39.55-47.83)[f]	71.91 (63.25-80.51)	64.46 (53.03-75.03)	72.24 (63.96-80.54)[f]
Age, years	60 (56-64)[d]	60 (56-64)	48 (48-48)	59 (55-64)[e]	62 (58-65)	56 (52-59)[g]	56 (53-60)	64 (59-66)	56 (52-59)[g]
Lightest skin colour	4 (2-8)[d]	4 (2-8)	2 (2-2)	5 (3-9)	4 (2-8)	6 (5-10)[f]	6 (4-9)	2 (1-7)	6 (4-9)[g]
Darkest skin colour	11 (8-12)[d]	11 (8-12)	13 (13-13)	11 (8-13)[e]	9 (6-12)	13 (11-14)[f]	12 (11-14)	9 (7-11)	12 (11-14)[g]
Delta skin colour[b]	5 (3-6)	5 (3-6)	11 (11-11)[f]	5 (3-7)	5 (4-6)	4 (3-8)	6 (3-7)	5 (4-7)	6 (3-7)

Data as median (Q25-Q75)
[a]25(OH)D conversion factor: nmol/l x 0.40 = ng/ml (Vitamin D3 (25-OH) [package insert])
[b] Delta skin colour/the changes of the skin colour = Darkest skin colour – Lightest skin colour
[c]Significantly different from vitamin D insufficiency,p<0.0167 (Mann-Whitney U tests with Bonferroni correction)
[d]Significantly different from vitamin D sufficiency, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)
[e]Significantly different from vitamin D sufficiency, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)
[f] Significant difference between groups at p<0.05
[g] Significant difference between groups at p<0.001

Table 4.22 illustrates dietary vitamin D and calcium intake in the urban and rural population according to vitamin D status. There were no significant differences in the dietary vitamin D and calcium intake in urban and rural women between each categories of vitamin D status. Although there was no significant difference, women with sufficient vitamin D (5.27 µg/day) had higher consumption of dietary vitamin D compared to those women with insufficient vitamin D (4.74 µg/day) or who were deficient (3.55 µg/day). A higher intake of dietary calcium also had been found in women with sufficient vitamin D (523 mg/day) than those with insufficient vitamin D (420 mg/day) and those deficient (420 mg/day).

Table 4.22 : Dietary vitamin D and calcium intake among urban and rural women according to vitamin D status

	Vitamin D deficiency (<30 nmol/L)			Vitamin D insufficiency (30-50 nmol/L)			Vitamin D sufficiency (≥50 nmol/L)		
Parameters	Overall, n=48	Urban, n=47	Rural, n=1	Overall, n=74	Urban, n=40	Rural, n=34	Overall, n=278	Urban, n=20	Rural, n=258
Dietary vitamin D intake, µg/day	3.55 (2.57-7.43)	3.58 (2.56-7.69)	2.82 (2.82-2.82)	4.74 (3.09-7.32)	4.84 (2.54-7.40)	4.71 (3.82-7.39)	5.27 (3.14-8.56)	5.52 (3.26-7.40)	5.24 (3.14-8.59)
Dietary calcium intake, mg/day	420 (306-608)	416 (305-604)	737 (737-737)	420 (319-621)	388 (328-606)	485 (300-631)	523 (333-736)	463 (360-535)	533 (330-758)

Data as median (Q25-Q75)

Table 4.23 displays sun exposure levels in urban and rural women according to vitamin D status. Overall, women with sufficient vitamin D (7.88 hours/week) had significantly higher hours of sun exposure per week compared to those women with insufficient vitamin D (4.05 hours/week) and those deficient (3.38 hours/week) respectively. Women with sufficient vitamin D (0.94) also had significantly higher sun exposure index than those women with insufficient vitamin D (0.59) or deficient (0.76) respectively. On the other hand, women with sufficient vitamin D (12%) had a significantly lower fraction of BSA exposure to the sun than women with insufficient vitamin D (18%) and those deficient (21%) respectively. According to area, rural women had significantly higher hours of exposure to the sun per week compared to urban women in the vitamin D insufficient range (6.34 vs 2.29 hours/week, $p<0.001$) and sufficient (8.00 vs 3.42 hours/week, $p=0.016$). On the other hand, rural women with insufficient vitamin D (0.13 vs 0.21%, $p<0.001$) or sufficient (0.11 vs 0.34%, $p<0.001$) groups had a significantly lower fraction of BSA exposure to the sun than urban women in that group. However, no significant differences were seen in the sun index between urban and rural women in all categories of vitamin D status.

Table 4.23 : Sun exposure among urban and rural women according to vitamin D status

	Vitamin D deficiency (<30 nmol/L)			Vitamin D insufficiency (30-50 nmol/L)			Vitamin D sufficiency (≥50 nmol/L)		
Parameters	Overall, n=48	Urban, n=47	Rural, n=1	Overall, n=74	Urban, n=40	Rural, n=34	Overall, n=278	Urban, n=20	Rural, n=258
Hours of sun exposure per week	3.38 (1.02-4.68)[b]	3.33 (1.00-4.67)	5.00 (5.00-5.00)	4.05 (1.41-8.35)[c]	2.29 (1.17-4.15)	6.34 (4.02-13.80)[e]	7.88 (3.50-14.86)	3.42 (2.18-9.33)	8.00 (3.63-16.27)[d]
Fraction of BSA exposed to the sun	0.21 (0.18-0.37)[b]	0.21 (0.21-0.37)	0.14 (0.14-0.14)	0.18 (0.10-0.22)[c]	0.21 (0.13-0.44)	0.13 (0.07-0.17)[e]	0.12 (0.07-0.18)	0.34 (0.21-0.45)	0.11 (0.07-0.17)[e]
Sun Index[a]	0.76 (0.23-1.17)[b]	0.79 (0.23-1.17)	0.71 (0.71-0.71)	0.59 (0.30-1.21)[c]	0.54 (0.22-1.10)	0.78 (0.41-1.77)	0.94 (0.42-1.87)	1.35 (0.70-2.69)	0.91 (0.42-1.86)

Data as median (Q25-Q75)

[a] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight

[b] Significantly different from vitamin D sufficiency,$p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[c] Significantly different from vitamin D sufficiency,$p<0.0167$ (Mann-Whitney U tests with Bonferroni correction)

[d] Significant difference between groups at $p<0.05$

[e] Significant difference between groups at $p<0.001$

4.13 Correlations between Serum Vitamin D and Other Variables

4.13.1 25(OH)D and physical/biochemical measurement

Table 4.24 shows the correlation between 25(OH)D and physical/biochemical parameters. Age and household income was found to be negatively associated with serum vitamin D. On the other hand, waist circumference, darkest skin colour and delta skin colour was positively associated with serum vitamin D. However, no correlation was found between BMI, body fat percentages, visceral fat, lightest skin colour and serum i-PTH with serum vitamin D.

Table 4.24 : Bivariate correlation between 25 (OH) Vitamin D3 and physical/biochemical parameters

	25 (OH) Vitamin D3	
	Spearman's rho	p-value
Age	-0.028**	<0.001
Household income	-0.224**	<0.001
BMI	0.058	0.248
Waist circumference	0.114*	0.023
Body fat percentages	-0.094	0.061
Visceral fat	0.037	0.457
Darkest skin colour	0.137**	0.006
Lightest skin colour	0.062	0.212
Delta skin colour[a]	0.115	0.022
PTH	-0.048	0.342

Data as Spearman's rho
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).
[a] Delta skin colour/the changes of the skin colour = Darkest skin colour – Lightest skin colour

4.13.2 25(OH)D and sun exposure

Table 4.25 displays the correlation between the duration of sun exposure per week, the fraction of BSA exposure to the sun and calculated sun index to the 25(OH)D levels. Hours of sun exposure per week and sun index correlated positively with 25(OH)D levels. Conversely, a fraction of BSA exposure to the sun negatively correlated with 25(OH)D levels.

Table 4.25 : Bivariate correlation between 25 (OH) Vitamin D3 and sun exposure

	25 (OH) Vitamin D3	
	Spearman's rho	p-value
Hours of sun exposure per week	0.342**	<0.001
Fraction of BSA exposed to sunlight	-0.264**	<0.001
Sun Index[a]	0.180**	<0.001

Data as Spearman's rho
[a] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight
** Correlation is significant at the 0.01 level (2-tailed).

4.13.3 25(OH)D and vitamin D and calcium intake

Table 4.26 presents the correlation between vitamin D and calcium intake and serum vitamin D. There was no association between vitamin D intake and serum vitamin D level. On the other hand, calcium intake was significantly associated with serum vitamin D.

Table 4.26 : Bivariate correlation between 25 (OH) Vitamin D3 and Vitamin D and calcium intake

	25 (OH) Vitamin D3	
	Spearman's rho	p-value
Dietary vitamin D intake	0.084	0.095
Dietary calcium intake	0.162**	0.001

Table 4.26, continued.

Data as Spearman's rho
** Correlation is significant at the 0.01 level (2-tailed).

4.14 Factors Predicting Serum Vitamin D Concentrations

Table 4.27 presents the factors predicting serum vitamin D concentration assessed by stepwise linear regressions. In a stepwise linear regression, all measured factors that were significantly associated with 25(OH)D concentrations in univariate analyses ($p<0.05$) were included in the model. There were no incidence of multicollinearity problems in this model since neither of the predictor variables had a VIF greater than ten. This model gave 43.8% of the variance (adjusted R^2). The factors that were significantly related to 25(OH)D concentrations were rural dwelling, being Chinese, hours of sun exposure per week and use of vitamin D containing supplements. Rural women have 37.09 nmol/L higher vitamin D concentrations than urban women. Moreover, Chinese women have 9.72 nmol/L higher 25(OH)D concentration compared to Malay women. In addition, 25(OH)D levels increased by 0.29 nmol/L for every unit increment in hours of sun exposure per week. Furthermore, women who take vitamin D containing supplements have 6.11 nmol/L higher 25(OH)D levels than those women who did not take vitamin D containing supplements. There was no significant relationship between 25(OH)D concentrations and age (p=0.911), household income (p=0.503), dietary calcium intake (p=0.062), fraction of BSA exposure to the sun (p=0.899), sun index (p=0.926), waist circumference (p=0.964), darkest (p=0.703) and delta changes (p=0.349) of the skin colour, and Indians (p=0.207).

Table 4.27 : Factors predicting serum vitamin D concentration assessed by stepwise linear regressions

	Serum 25(OH)D, nmol/L		
	Unstandardized coefficient[a]	Standardized coefficient[a]	p-value
Area[b]	37.09 (32.00, 42.18)[c]	0.719	<0.001
Race, Chinese[d]	9.72 (4.16, 15.27)	0.157	0.001
Hours of sun exposure/week	0.29 (0.10, 0.48)	0.124	0.002
Use of vitamin D containing supplements[e]	6.11 (0.39-11.83)	0.087	0.036

[a] β coefficient
[b] Area, 0 for urban, 1 for rural
[c] 95% CI in parentheses (all such values)
[d] Race, Chinese, 0 for others, 1 for Chinese
[e] Vitamin D containing supplements, 0 for no, 1 for yes

CHAPTER V

5.0 DISCUSSION

The aim of this study is to determine the vitamin D status of urban and rural women in Malaysia and factors predicting 25(OH)D levels. According to Chan et al. (2009), urban and rural Malaysians have different concentrations of vitamin D; rural subjects had higher concentrations of vitamin D compared to urban subjects [70.7±18.25 vs 45.73±15.50 nmol/L (or 28.28±7.3 vs 18.29±6.2 ng/ml), $p<0.0001$]. The present study is in agreement with previous findings reported by Harinarayan, et al. (2007) which showed that rural women in India had significantly higher mean of vitamin D levels than urban women [47.5 vs 38.8 nmol/L (19 vs 15.5 ng/ml), $p<0.001$]. Another study by Chailurkit et al. (2011) also found that both male (88.9 vs 78.4 nmol/L, $p<0.001$) and female (75.8 vs 66.6 nmol/L, $p<0.001$) subjects residing in the rural municipal area of Thailand had significantly higher vitamin D levels than urban counterparts.

The cut-off point of vitamin D status remains controversial. Two levels of 25(OH)D which are <75 or <50 nmol/L (<30 or <20 ng/ml) are normally used to define vitamin D insufficiency (Dawson-Hughes, et al. 2010). As reported by the Institute of Medicine (IOM) in 2010, vitamin D concentrations of ≥50 nmol/L (or ≥20 ng/ml) contributed to the requirements of at least 97.5% of the population (Ross, et al. 2011). In addition, a global map was developed by the International Osteoporosis Foundation (IOF) and DSM Nutritional Products to demonstrate vitamin D status. Vitamin D concentrations were classified as >75 as optimal, 50-74 as suboptimal, 25-49 as insufficient, and <25 nmol/L as deficient respectively (DSM Nutritional Products, 2012). Moreover, Vitamin D deficiency and insufficiency was defined as 25(OH)D below 50 nmol/L (20 ng/ml)

and 52.5-72.5 nmol/L (21–29 ng/ml) respectively by the US Endocrine Society Clinical Practice Guideline (Holick, et al. 2011). However, vitamin D levels of >75 nmol/L (>30 ng/ml) are not consistently associated with increased benefits (Ross, et al. 2011). Consequently, most researchers determine vitamin D insufficiency as 25(OH)D<50 nmol/L (Zittermann, et al. 2010).

Using the cut-off points defined by IOM (Ross, et al. 2011; Institute of Medicine, 2011), the present study found that higher percentages of urban women (43.9%) had vitamin D deficiency (<30 nmol//L) than rural women (0.3%). On the other hand, 18.7% of urban women and 88.1% of rural women were vitamin D sufficient. However, the present study found a high prevalence (81.3%) of urban women had vitamin D <50 nmol/L than what was reported in previous study (Suriah A Rahman, et al. 2004) regardless of the vitamin D assay used. Foong et al. (2011) also reported a high prevalence (87%) of urban females in Kuala Lumpur with vitamin D level <50 nmol/L.

The prevalence of vitamin D deficiency/insufficiency had been reported previously in Asian regions. For example, vitamin D deficiency (<50 nmol/L or <20 ng/ml) had been seen in 44% and 62% of rural and urban subjects in Tirupati, India (Harinayan, et al. 2007). Using data from the Thai 4th National Health Examination Survey, vitamin D insufficiency [25(OH)D <50 nmol/L] was found in 14.3%, 6.5%, 4.3%, 2.8% and 6.3% in Bangkok, Central, North, Northeast and South of Thailand (Chailurkit, et al. 2011). In addition, a high prevalence of vitamin D insufficiency (<50 nmol/L) had been reported in Bangladeshi female adults (88.5%) (Islam, et al. 2008). The prevalence of women of child-bearing age in Hong Kong and Beijing who had vitamin D insufficiency (≤50 nmol/L) is even higher; is more than 90% (Woo, et al. 2008). Using ECLIA Vitamin D3 (25-OH) to measure vitamin D status, which is the same assay used

in the present study, Ho-Pham et al. (2011) reported that 40% and 56% of Vietnamese women aged between 30-60 and >60 years had vitamin D insufficiency [25(OH)D<75 nmol/L or <30 ng/ml]. The present study shows that 47.1% and 52.9% of urban women aged ≤60 and >60 years had vitamin D <75 nmol/L respectively, where the prevalence is consistent with what was reported earlier (Ho-Pham, et al. 2011).

Contrary to existing evidence, that shows an inverse correlation between serum vitamin D and PTH concentration (Suriah A Rahman, et al. 2004; Harinarayan, 2005; Ho-Pham, et al. 2011), the present study has not found such correlation between serum intact PTH and 25(OH)D levels. This result was consistent with those of Elsammak et al. (2011) that reported PTH concentrations were not associated with serum vitamin D levels in male and female subjects (Elsammak, et al. 2011). Binkley et al. (2007) also did not find the relationship between serum 25(OH)D and PTH. As a result, PTH concentrations should not be used clinically as an indicator of vitamin D deficiency (Binkley, et al. 2007). As such, vitamin D levels should be determined if vitamin D deficiency/insufficiency is suspected, regardless of the calcium and PTH value (Elsammak, et al. 2011).

Ho-Pham et al. (2011) stated that the relationship between serum 25(OH)D and PTH can be used as a threshold of 25(OH)D to determine vitamin D deficiency. For instance, Islam et al. (2008) who plotted an exponential curve to explore the relationship between serum 25(OH)D and PTH reported that the plateau for the serum PTH concentrations was at 21 ng/L and 25(OH)D >38 nmol/L kept low concentrations of serum PTH since the curve fitted well. Conversely, since there was none of the spline regression model that fitted better than the simple linear regression model, the writers concluded that there was no cut-off point of vitamin D concentrations at which PTH concentrations

plateau (Ho-Pham, et al. 2011). The present study found a poor relationship between serum PTH and vitamin D in urban and rural subjects. The non-linear curve also did not plateau. The possible explanation is the majority of subjects who had vitamin D deficiency or insufficiency had normal PTH values. Furthermore, it is difficult to have an actual value of PTH since its molecules which are unstable in nature, make it easily broken down in the blood after venipuncture (Sturgeon, et al. 2011).

Steingrimsdottir et al. (2005) reported that subjects who had vitamin D levels >45 nmol/L (>18 ng/ml) had the lowest concentration of PTH but subjects who had vitamin D level <25 nmol/L (<10 ng/ml) had the highest concentration of PTH. Another study conducted in postmenopausal south Indian women, illustrated that subjects who had severe vitamin D deficiency [25(OH)D<12.5 nmol/L or <5 ng/ml] (47±34 pg/ml) and moderate vitamin D deficiency [25(OH)D between 12.5-25 nmol/L or 5-10 ng/ml] (26±14 pg/ml) had significantly higher levels of PTH than subjects who had normal vitamin D [25(OH)D>50 nmol/L or 20 ng/ml] (19±10 pg/ml) (Harinarayan, 2005). The present study also found that the PTH level was highest in urban women who had vitamin D deficiency and lowest in rural women who had vitamin D sufficiency.

The disparity in vitamin D status between urban and rural groups could be explained by skin colour, sun exposure or vitamin D and calcium intake. Greater melanin content in those people with darker skins inhibits the cutaneous synthesis of vitamin D (Meer, et al. 2008) by more than 90% (Bodnar, et al. 2007), which leads to poor vitamin D levels. This is because melanin absorbs electromagnatic radiation and competes with 7-dehydrocholesterol for UVB photons (Mallah, et al. 2011). As such, persons with higher melanin content or darker skin pigmentation need longer sun exposure compared to those with lighter skin pigmentation to produce an equivalent amount of vitamin D3

(Tsiaras, et al. 2011). The present study showed that hours of sun exposure positively correlated with darker skin colour (rho=0.121, p=0.015) (data not shown) suggesting that darker skin colour is a sign of sunlight exposure (Rockell, et al. 2008). Although rural women in the present study had darker skin than urban women, they spent a lot of time under the sun, leading to a higher levels of serum 25(OH)D as darkening of exposed skin was associated to the hours of time spent outdoors (Binkley, et al. 2007).

Binkley et al. (2007) who had studied the vitamin D status in 93 adults in Hawaii reported that there was no correlation between serum 25(OH)D and lightest, darkest and delta skin colour. A study conducted by Hall et al. (2010) showed that serum 25(OH)D correlated with the colour on the back of the hands (r=0.39; p=0.0008) and inner arm (r=0.29; p=0.0136). The present study found that there was positive correlation between serum 25(OH)D levels and darker and delta skin colour. This study also showed that women with darker skin colour tend to have vitamin D sufficiency. Higher sunlight exposure causes the colour of the exposed skin get darker, resulting in higher levels of serum 25(OH)D. On the other hand, lighter skin colour of the unexposed skin results in higher vitamin D levels (Rockell, et al. 2008). However, the present study did not find the significant correlation between serum 25(OH)D and light skin colour. This finding is consistent with Rockell et al. (2008).

Skin colour varies between ethnicities (Rockell, et al. 2008). The vitamin D level is consequently, influenced by ethnicity. The lower concentrations of vitamin D is normally experienced by those ethnic groups with darker skin colour compared to ethnic groups with lighter skin colour (Rockell, et al. 2008). For example, white pregnant women at 4-21 weeks of gestation had significantly higher serum 25(OH)D levels compared to black pregnant women (73.1 vs 40.2 nmol/L, p<0.001) (Bodnar, et al.

2007). Similarly, white men in Boston had higher levels of vitamin D [93.5 ± 35 nmol/L (37.4 ± 14.0 ng/ml)] compared to Blacks [62.5 ± 36.8 nmol/L (25.0 ± 14.7 ng/ml)] and Hispanic men [82.3 ± 34.8 nmol/L (32.9 ± 13.9 ng/ml)] (Hannan, et al. 2008). In Malaysia, higher melanin concentrations among Malays compared to Chinese women might be a reason for the significantly lower level of 25(OH)D in Malay than Chinese postmenopausal women (44.4 ± 10.6 vs 68.8 ± 15.7 nmol/L) (Suriah A Rahman, et al. 2004). Another study conducted in women aged between 18-40 years showed that the Chinese (58 nmol/L) had higher concentrations of vitamin D compared to Malay (43 nmol/L) and Indian (45 nmol/L) women (Green, et al. 2008). In contrast, Malay women in the present study had significantly higher serum 25(OH)D levels compared to the Chinese and Indians. This might be due to the higher sun exposure and dietary vitamin D and calcium intake in Malays than Chinese and Indian women. In contrast with urban Malays who were less active outdoors and lack sun exposure (Suriah A Rahman, et al. 2004), Malay women in the present study, with high number of them staying in rural areas, were more likely to spend more time under the sun, resulting in higher levels of vitamin D. Malay women in the overall population had also a higher intake of dietary vitamin D food than Chinese and Indian women. These findings are consistent with Malay females above 50 years who consume higher amount of vitamin D compared to Chinese and Indians (Lee, 2011). However, according to area (urban vs rural), urban Chinese have significantly higher level of serum 25(OH)D compared to urban Malays [36.23 (27.95-49.68) vs 27.08 (19.83-36.50) nmol/L] respectively. Moreover, rural Chinese have significantly higher concentrations of vitamin D compared to rural Indians [78.65 (66.10-92.73) vs 63.23 (50.90-72.25) nmol/L] respectively.

As stated above, rural women spent more time under the sun, but had less BSA exposure to the sun due to the style of clothing i.e. outfits which were more covered-up.

However, the sun index was significantly higher in rural women. Higher percentages of Malay women staying in rural areas, who are Muslims and following their cultural practices in terms of style of clothing, limit their BSA exposure when being outdoors. However, the duration spent outdoors was greater, resulting in higher vitamin D levels. Most rural women were housewives, who reported that they spent a lot of time performing outdoor activities such as hanging out clothes, sweeping the porch or compound and gardening. In addition, rural women who worked were more likely to work outdoors compared to urban women who worked indoors most of the time.

Harinarayan et al. (2008) found that male and female adults from rural Andhra Pradesh had significantly higher 25(OH)D concentrations compared to those subjects staying in urban areas. Rural subjects who work as agricultural laborers spent about 8 hours per day under the sun and had higher BSA sun exposure. On the other hand, urban subjects mainly works indoors between 10.00 am to 5.00 pm with only the face and forearm exposed to the sun. The same goes for Bangladeshi females who had low serum vitamin D levels (36.7 nmol/L) due to limited exposure to the sun as they worked inside a garment factory for 14–16 hours every day (Islam, et al. 2008).

Fewer hours of sun exposure per week were seen in those groups who had vitamin D deficiency compared to those women in the vitamin D sufficient group. These findings are consistent with those reported by Zargar et al. (2007) whereby subjects who had vitamin D deficiency [25(OH)D<50 nmol/L) spent significantly less time in the sun per week compared to subjects who had vitamin D sufficiency [(15.6±8.2 vs 20.6±6.5) hours per week; $p<0.03$]. According to Islam et al. (2008), spending 2-3 times a week under the sun for about 10-15 minutes (i.e. 20 to 45 minutes per week) results in sufficient vitamin D production by the skin. Rural women in the present study spent

about 2.92 hours per week under the sun with 21% of BSA exposure, but they still had low levels of serum 25(OH)D. The possible explanation was overestimation of sun exposure, obstruction of sunshine by tall buildings, air pollution (Islam, et al. 2008), and the texture as well as style and colour of clothing (Mallah, et al. 2011).

With regard to the application of sunscreen, urban women were more likely to use sunscreen when exposed to the sun than rural women. The cutaneous production of vitamin D is suppressed with the use of sunscreen with Sun Protection Factor (SPF) 8 (McCarty, 2008). Moreover, the photoconversion of 7-dehydrocholesterol to previtamin D3 is disrupted by 5% para-aminobenzoic acid (PABA) in sunscreen (Tsiaras, et al. 2011). As reported by Norval et al. (2009), subjects who use PABA sunscreen on the exposed parts of the body before being outdoors have half point lower concentration of vitamin D compared to the group who did not apply sunscreen.

The significantly higher level of serum vitamin D in rural women compared to urban women can also be explained by the dietary intake of vitamin D. At present, rural women had significantly higher dietary intake of vitamin D compared to urban women. Higher consumption of vitamin D was also seen in pregnant women from the rural area compared to urban pregnant women in northern Indian (16.5±7.7 vs 16.4±7.4 IU/d) (Sachan, et al. 2005). Dietary vitamin D intake by urban women in the present study was a bit lower compared to those reported by Lee (2011) in free living men and women aged above 50 years who consume 5.3 µg/day of vitamin D food. The present study also showed that fish, oily fish and shellfish was the highest contributor of vitamin D in rural women. Dark fish such as salmon, sardines and mackerel contain an even higher concentrations of vitamin D. As a result, frequent intake of fish was found to be significantly related to the higher concentrations of serum vitamin D in Korean adults

(Lym, et al. 2009). Among urbanites, most vitamin D food comes from milk and milk products. Similarly, milk and milk products were the major vitamin D food consumed by Malays (37%) and Indians (55%) in Klang Valley (Lee, 2011).

Most urban and rural women did not meet the RNI for vitamin D intake. This result was in agreement with another study, where only 12% of 60 urban adults in Malaysia met the RNI for vitamin D (Lee, 2011). In Taiwan, the percentage of women aged above 45 years who meet the RNI for vitamin D from food sources alone was in the range of 45.9-74.2% (Lee, et al. 2008). This value is higher compared to those obtained from the present study [10.8% (overall population)] (data not shown) probably because the Taiwan population depends on food sources instead of sun exposure to achieve adequate levels of vitamin D (Lee, et al. 2008). Those who meet RNI for vitamin D had significantly higher median levels of serum 25(OH)D compared to those who did not meet RNI for vitamin D [65.10 (48.53-76.10 vs 64.08 (45.54-75.31 µg/day, p=0.040] (data not shown). Unlike studies showing a positive correlation between dietary vitamin D intake and serum 25(OH)D (Burgaz, et al. 2007; Hall et al. 2010), the present study did not find significant correlation between dietary vitamin D intake and serum 25(OH) vitamin D level. This is similar to that reported by Puri et al.(2008). The lack of correlation between dietary vitamin D intake and 25(OH)D levels might be due to the limited database of Malaysian food which reduces the accuracy of the estimation of vitamin D consumption. In terms of vitamin D intake among different groups of vitamin D status, those women in vitamin D sufficiency groups had higher dietary intake of vitamin D compared to those in vitamin D deficiency and sufficiency groups, although no significant differences found.

Harinarayan et al. (2004) reported that urban Tirupati had significantly higher dietary intakes of calcium than rural Tirupati (356±5.0 vs 264±1.94 mg/day) but the serum 25(OH)D concentrations of urban Tirupati was significantly lower compared to rural Tirupati (33.8±1.48 vs 52.5±1.15 nmol/L or 13.52±0.59 vs 21±0.46 ng/ml). Conversely, rural women in the present study had higher serum vitamin D levels in accordance with the higher consumption of calcium food compared to urban women. The calcium intake of urban women in the present study was 423.49 mg/day, a bit lower than what was previously seen in postmenoupausal women in Kuala Lumpur (498.7 mg/day) (Chee, et al. 2002), but higher than non-pregnant women aged between 18-40 years in Kuala Lumpur (386 mg/day) and Jakarta (270 mg/day) and also elderly women in Bangkok (322 mg/day) (Kruavit, et al. 2012). Dietary calcium plays an important role in skeletal health. It also reduces the risks to getting osteoporosis and fractures (Magkos, et al. 2006). On the other hand, 25(OH)D will be converted to its polar metabolites in the liver, resulting in secondary vitamin D deficiency in individuals with low intake of calcium. In addition, PTH concentrations will be increased with low consumption of calcium, leading to the conversion of 25(OH)D to $1,25(OH)_2D$ which in turn enhances the intestinal calcium absorption (Harinarayan, et al. 2007; Harinarayan, et al. 2008).

Zargar et al. (2007) who had studied the vitamin D status of healthy adults in India reported that subjects who had vitamin D deficiency had lower consumption of calcium per day compared to those without vitamin D deficiency (306±84.4 vs 444±46.3 mg/day, $p<0.01$). The present study showed that the dietary calcium intake of those women in the vitamin D sufficiency group was higher compared to those who had vitamin D deficiency, however, no significant difference found. This differs from those obtained by Harinarayan (2005) who found that dietary calcium intake was significantly higher in those subjects who had moderate vitamin D deficiency (12.5-25 nmol/L or 5-

10 ng/ml) than subjects who had normal vitamin D status (>50 nmol/L or 20 ng/ml) (346±64 vs 299±55 mg/day) respectively. The highest percentage of calcium food sources in Chinese postmenopausal women in Kuala Lumpur was vegetables and bean (32%) and followed by dairy products (26%) (Chee, et al. 2002). In contrast, milk and milk products was the highest percentage of calcium food sources for both urban and rural women in the present study.

Chee et al. (2002) documented that 3.9% of Chinese postmenopausal women residing in Kuala Lumpur had calcium intake above 800 mg/day; 56% of subjects did not meet the Malaysian RDA requirement for calcium, which is 450 mg/day. In another study conducted with healthy urban and rural Malaysian adults reported that less than 2/3 of women in both area met the RDA for calcium (Chee, et al. 1997). Sachan et al. (2005) who conducted study in urban and rural pregnant women in India found that 72% of urban women and 88% of rural women did not met the RDA for calcium (<1200 mg/day). Most of the subjects in the present study also did not meet RNI for calcium. The percentages of rural women who met the RNI for calcium was much lower compared to those reported by Suzana Shahar et al. (2007) in elderly rural Malays. The percentage of urban women who met the RNI for calcium was also lower (0.9%) than those seen in post-menopausal Chinese in Kuala Lumpur, where only 3.4% of them meet the RNI for calcium from food sources (Chee, et al. 2010).

The present study contradicts the existing evidence where higher vitamin D levels were achieved among subjects who took supplements containing vitamin D compared to those subjects who did not take vitamin D containing supplements (Lips, et al. 2006; Burgaz, et al. 2007). For example, Lips et al. (2006) found that subjects who took vitamin D containing supplements regularly have 17% higher levels of vitamin D

compared to those who did not (78 vs 67 nmol/L, p=0.06) respectively. In another study conducted in osteoporotic postmenopausal women in 18 countries with latitudes ranging 64N–38S, showed that women who took vitamin D supplements of 10 μg/day (≥400 IU daily) (52.4%) were less likely to get vitamin D <75 nmol/L (<30 ng/ml) compared to those who did not (70.5%). The same observation also was made if using <22.5 nmol/L (<9 ng/ml) (1.6% vs 3.5%) or <50 nmol/L (<20 ng/ml) (20.1% vs 37.1%) as cut-off points of 25(OH)D (Lips, et al. 2006). However, when urban and rural women were analyzed separately, it showed that each urban (35.80 vs 31.11 nmol/L, p=0.372) and rural (74.34 vs 69.45 nmol/L, p=0.338) women in the present study who took vitamin D containing supplement had higher serum value 25(OH)D compared to those who did not take vitamin D containing supplement, although no significant difference was seen (data not shown). Urban women (32.7%) in the present study were more prone to use vitamin D containing supplement than rural women (4.1%). Overall, 11.8% of the subjects took vitamin D containing supplements (data not shown). Similarly, only 13% of osteoporosis patients in Malaysia take both calcium and vitamin D supplements, where 6% of them take it separately and 7% of them take a combination of pills (Chan, et al. 2010).

A significant negative correlation was found between BMI (r=-0.27, p<0.001) and fat mass (r=-0.24, p<0.001) with serum vitamin D levels (Suriah A Rahman, et al. 2004). BMI also was reported to be significantly associated with vitamin D insufficiency (<50 nmol/L) (Moy, et al. 2011). Since vitamin D is fat soluble (Moy, et al. 2011) it tends to deposit in adipose tissue in obese people, leading to decreasing bioavailability from cutaneous vitamin D (Suriah A Rahman, et al. 2004; Moy, et al. 2011). In obese individuals, vitamin D endocrine system is altered, which increases production of $1,25(OH)_2D$. As a result, it reduces the vitamin D levels (Harris, et al. 2007) via

negative feedback control on hepatic synthesis (Suriah A Rahman, et al. 2004; Harris, et al. 2007). The present study did not find significant association between BMI and body fat percentage and vitamin D levels. This might be due to the high prevalence of the overweight and obese [(BMI≥23 kg/m^2) (WHO expert consultation, 2004)] (86.3%), in this group of women with median BMI of 27.2 (24.9-30.1) kg/m^2 (data not shown).

There was significantly negative correlation between age and serum 25(OH)D levels, where younger subjects had higher levels of serum vitamin D. The results are in agreement with Suriah A Rahman, et al. (2004). It follows that subjects who were younger were found to have vitamin D sufficiency compared to the older ones who were more likely to have vitamin D deficiency. Older people tend to have vitamin D deficiency because reduced synthesis of vitamin D by the skin (Lee, et al. 2008; Aldasouqi, et al. 2011; Kruavit, et al. 2011) which goes up to 75% by the age of 70 (Kruavit, et al. 2011). In addition, less sunlight exposure also contributes to the vitamin D deficiency in the elderly (Lee, et al. 2008; Kruavit, et al. 2011). In the present study, a poor negative correlation was found between age and hours of sun exposure per week (rho=-0.298, $p<0.001$) suggesting that time spent under the sun was lower with advancing age (data not shown). Unlike elderly Thais who have more leisure time after retirement and spend more time in the sun (Chailurkit, et al. 2011), older women in the present study were more likely to be indoors compared to the younger women who were more likely to spend a longer time under the sun. The older people therefore need an adequate intake of vitamin D to achieve sufficient levels of vitamin D (Lee, et al. 2008). Decreasing appetite (Aldasouqi, et al. 2011), a vegetarian lifestyle (Whiting, et al. 2005), and avoidance of vitamin D food, especially food which is high in cholesterol content such as animal skin, eggs, offal and certain types of seafood (e.g. shrimps) (Lee, et al. 2008) will further lead to a reduction of vitamin D intake. Although there was no

significant correlation between age and dietary vitamin D intake in the present study, it was shown that dietary vitamin D intake was reduce with increasing age (rho=-0.011, p=0.832) (data not shown).

In the stepwise linear regression, area, being Chinese, hours of sun exposure per week and use of vitamin D containing supplements were the significant predictors of vitamin D. Residing in a rural area was significantly related to the increment of vitamin D concentrations. These findings were consistent with those of Gannage´-Yared et al. (2000) who discovered that the location or dwelling is a significant predictor of vitamin D; rural subjects had higher levels of vitamin D compared to those subjects in urban areas. This is probably because rural subjects work in the fields and spend a lot of time under the sun (Gannage´-Yared, et al. 2000). In contrast, Chailurkit et al. (2011) claimed that the lowest vitamin D levels seen in subjects residing in Bangkok, the largest city in Thailand, could be due to air pollution. Tropospheric ozone which is air pollution normally seen in urban areas, causes an efficient absorber of UVB radiation, reduces the skin synthesis of vitamin D resulting in low vitamin D levels (Gannage´-Yared, et al. 2000; Chailurkit, et al. 2011).

Being Chinese also one of the significant predictors of vitamin D. Similarly, Suriah A Rahman et al. (2004) found that race was significantly related to serum 25(OH)D concentration. The same goes to Meer et al. (2008) and Heere et al. (2010) who reported that ethnicity was the main factor influencing serum vitamin D levels. In Singapore, the odds for having low 25(OH)D levels (<20 μg/L) among Malays and Indians was 3.5 and 7.1 times higher than Chinese respectively. In addition, the prevalence of having vitamin D deficiency was higher among Indians and Malays than Chinese (Hawkins, 2009). Furthermore, higher percentages of Indian (68%) and Malay (74%) women aged

18–40 years in Kuala Lumpur experienced vitamin D insufficiency [25(OH)D<50 nmol/L] compared to Chinese women (38%) (Green, et al. 2008). The present study also found a high prevalence of urban Malay (95.2%) and Indian (83.3%) women had vitamin D insufficiency than Chinese (75.0%) women. Higher melanin content among the Malay than Chinese women might be a reason for developing vitamin D deficiency (Suriah A Rahman, et al. 2004). Moreover, the increase in the 24-hydroxylase activity in Indians has resulted in the decline of 25(OH)D levels (Hawkins, 2009).

The present study supported the previous finding (Foong, 2011) where hours of sun exposure was the predictor of vitamin D concentration. Jacobs et al. (2008) also reported that sun exposure (min/week) is one of the predictors of 25(OH)D concentrations (Jacobs, et al. 2008). In addition, men and women from Vietnam tend to have vitamin D insufficiency [25(OH)D<75 nmol/L or 30 ng/ml] with hours of sun exposure of less than 10 hours/week (Ho-Pham, et al. 2011). In the study of vitamin D status among healthy adults in India reported that subjects who had vitamin D sufficiency [25(OH)D≥50 nmol/L] had significantly longer hours of weekly sun exposure compared to those in the vitamin D deficiency (<50 nmol/L) group (20.6±6.5 vs 15.6±8.2 hours, p<0.03) respectively (Zargar, et al. 2007).

The present study also found that vitamin D containing supplement was the significant contributor of vitamin D levels. This is in accordance with the findings reported earlier (Brot et al. 2001; Burgaz et al. 2007; Meer et al. 2008; Absoud et al. 2011). A study conducted by Brot et al. (2001) on healthy perimenopausal Caucasian women aged between 45 – 58 years found that use of vitamin D supplements increased serum 25(OH)D by 15.7 nmol/L. Similarly, Burgaz et al. (2007) found that vitamin D levels increased by 11.8 nmol/L with regular consumption of dietary supplements containing

vitamin D. In addition, Meer et al. (2008) reported that use of vitamin D supplements was one of the contributors of vitamin D levels in a multiethnic population in the Netherlands. In the logistic regression, children in Great Britain who were not on vitamin D containing supplements had 3.7 times higher odds of being vitamin D insufficient than those subjects who consume vitamin D supplements (Absoud et al. 2011).

Previously, vitamin D FFQs had been developed for use in America (Blalock, et al. 2004), Japan (Uenishi, et al. 2008), and Canada (Pritchard, et al. 2010). Vitamin D intakes derived from the FFQ significantly correlated to the vitamin D intake derived from the 24-HDRs. The Pearson Correlation value between FFQ and 24-HDRs was in the range of 0.32 to 0.49 (Nurul-Fadhilah, et al. 2012; Liu et al. 2013). Chee et al. (2002) reported that the number of days used in FFQ (two or more than four days dietary recall) might influence the strength of the questionnaire. For example, Pritchard et al. (2010) found a strong correlation ($r=0.89$, $p<0.05$) between FFQ and 5-Day Diet Record (5DDR) to assess vitamin D intake in Canadian subjects. The correlation value between FFQ and 7-day dietary record (7-DR) for vitamin D was from 0.55 to 0.607 (Barrat, et al. 2012; Wu, et al. 2009).

The nutrient intake value derived from FFQ is normally higher compared to those obtained from 24-HDRs (Liu, et al. 2013). This includes the value of vitamin D FFQ which seems to be higher than the dietary recalls as reported previously (Liu, et al. 2013; Nurul-Fadhilah, et al. 2012). The present study also showed that the median intake of vitamin D derived from FFQ was significantly higher compared to 24-HDRs. On the other hand, there was no significant difference between FFQ and 5DDR found by Pritchard et al. (2010). The subjects may have reported that they consume certain

kinds of food in the FFQ, but did not include that food in the 24-HDRs. In addition, a long list of food items might be the reason for overestimation of nutrient intake (Chee, et al. 2002). The subjects may have incorrectly reported the frequency of eating and/or the portion size of the serving, resulting in overestimation of the FFQ (Barrat, et al. 2012).

A correlation analysis is normally used for validation purposes. However, it is still subject to debate since it only determines the strength of association between two parameters, but not the agreement between those parameters. As a result, cross classification intake and Bland-Altman analysis were used to determine the agreement between the methods (Liu, et al. 2013). The present study revealed that the FFQ developed was able to place 73.9% of the subjects into the same or adjacent (same ± 1 quartile) quartile of intake for vitamin D intake and 6.3% was grossly misclassified. The percentages for correct classification for vitamin D in the previous study vary from 39.2-100% while gross misclassification varies from 2.4-9% (Barrat, et al. 2012; Liu, et al. 2013; Nurul-Fadhilah, et al. 2012; Pritchard, et al. 2010; Tokudome, et al. 2005). Specificity which classifies subjects with vitamin D intake below RNI was high (86.7%), but sensitivity which describes subjects with intake above RNI was low (44.4%).

CHAPTER VI

6.0 CONCLUSION, LIMITATION AND RECOMMENDATION

6.1 Conclusion

This study was conducted to determine the vitamin D status of urban and rural women in Malaysia as assessed by sun exposure, vitamin D and calcium intake, demographic and anthropometric measurement and yet to evaluate the factors predicting 25(OH)D levels.

Rural women had significantly higher levels of serum 25(OH)D compared to urban women. Most urban women tend to have vitamin D deficiency, while higher percentages of vitamin D sufficiency had been seen in rural women. The disparities in characteristics and lifestyle between urban and rural women were explored in order to investigate vitamin D discrepancies between these two groups of women. Although urban women had significantly lower BMI and skin colour score on the darkest and lightest part of their body compared to rural women, these were not sufficient enough to provide them with sufficient levels of serum 25(OH)D. The higher socioeconomic status of urban women compared to that of rural women also did not ensure the high levels of vitamin D in this group of women. The older the urban women the greater their tendency to have low levels of vitamin D compared to their rural women.

Regarding sun exposure, rural women had significantly higher hours of sun exposure/week and sun index, but had lower BSA exposure to the sun compared to urban women. Hence the null hypothesis was rejected. This indicated that BSA alone

could not guarantee sufficient levels of vitamin D if the time spent under the sun was limited. Hours of sun exposure per week and sun index showed significantly positive correlation with serum 25(OH)D, while significant negative correlation was found between the fraction of BSA exposure to sunlight with serum vitamin D levels. Again, the null hypothesis was rejected.

This study also increased information on dietary intake of vitamin D and calcium. Rural women had a higher consumption of vitamin D and calcium food compared to urban women. The null hypothesis was therefore rejected. In urban areas, most of the vitamin D food sources come from milk and milk products, while rural women had a higher consumption of fish and shellfish. Milk and milk products was the most popular calcium food sources for both urban and rural groups. An important finding was that the majority of urban and rural women did not meet RNI for vitamin D and calcium. The null hypothesis in that higher vitamin D intake was not significantly associated with 25(OH)D levels was accepted. On the other hand, null hypothesis was rejected since calcium intake showed significant association with 25(OH)D levels.

Most urban and rural women did not usually take vitamin D containing supplements. However, there was a higher percentage of urban women who did take vitamin D containing supplements than rural women. BMI and body fat percentages were not significantly associated with serum 25(OH)D levels in the present study. Due to this, the null hypothesis was accepted. On the other hand, age was negatively associated with serum vitamin D levels. In other words, vitamin D levels decreased across the age categories, as can be shown in the present study that women aged <50 years had significantly higher vitamin D levels than women aged 50-59, 60-69 and 70-79 years. In terms of racial disparity in vitamin D levels, Malay women from overall population had

significantly higher vitamin D levels than the Chinese and Indian women. Subsequently, the null hypothesis was rejected. However, when urban and rural was analyzed separately, the results showed that Chinese from urban areas had significantly higher vitamin D levels than the Malay urban. Rural Chinese also had significantly higher vitamin D levels than rural Indian.

In terms of age, no significant differences were found in the dietary vitamin D and calcium intake between each age group. Overall, women aged 60-69 and <50 years have higher dietary vitamin D and calcium intake than other age groups respectively. Hours of sun exposure per week shows a significant decreasing trend across the ages. According to ethnicity, Malay women had significantly higher dietary intake of vitamin D and calcium compared to Chinese and Indian women respectively. Similarly, Malay women had significantly higher hours of sun exposure per week than Chinese and Indian women. Subjects who were in the vitamin D sufficient groups was found to have higher hours of sun exposure per week, sun index, vitamin D and calcium intake, but were younger and had a lower fraction of BSA exposure to sunlight compared to those in vitamin D deficient and insufficient groups. Rural dwelling, being Chinese, hours of sun exposure per week and use of vitamin D containing supplements were the significant predictors of serum 25(OH)D levels.

The present study also provides a useful tool to evaluate vitamin D intake in urban and rural Malaysian women aged above 45 years. The dietary vitamin D intake measured by FFQ was higher compared to those obtained from 24-HDRs. The null hypothesis was then rejected. Correlation analysis also showed that there was an association between dietary vitamin D intake derived by FFQ and 24-HDRs. As such again, the null hypothesis was rejected. Though the FFQ was not a perfect vitamin D dietary

assessment tool, but it could classify an individual's vitamin D intake into quartiles, whereby high proportions of subjects being classified into the same or adjacent quartile of vitamin D intakes and only a small number (<10%) being grossly misclassified. It also provided a high percentages of specificity, but low sensitivity for categorizing subjects into RNI for vitamin D food as reported by National Coordinating Committee on Food and Nutrition (NCCFN), Ministry of Health Malaysia. The FFQ is simple and convenient, easy to monitor, low cost and requires a short time to analyze. It can also be used in a clinical setting for bone health research and for the assessment of vitamin D intake.

This was a cross-sectional study which focused on vitamin D status and related factors contributing to the serum 25(OH)D concentrations in Malaysian urban and rural women. The present research has added to the information about the status of vitamin D, sun exposure as well as dietary vitamin D and calcium intake in this population. Although there were some limitations, the findings from the current research might serve as a reference for future research. The information about the importance of vitamin D and an approach for appreciation of the vitamin D status, such as promoting adequate sun exposure and intake of vitamin D food should be disseminated to the public.

6.2 Limitation and Recommendation

The present research has a number of limitations:

- First, the study subject consisted of women aged above 45 years. Hence, the results may not reflect on women below this age, to men, adolescents and children in Malaysia.

- Second, the sample size of this study might be have insufficient power to reveal the relationship between serum 25(OH)D and some of the variables.
- Third, the information given by the subjects regarding sun exposure might be incorrect since it was based on the patient recall of sun exposure over the previous week.
- Fourth, since the Cosmetic Colour Ruler has only a 16 point scale, skin colour that is darker than this could not be measured accurately and was assumed as being 16. It is recommended that future research using proper measurement tools to measure skin colour is needed.
- Fifth, since there is a lack of database on Malaysian food, the assessment of vitamin D intake has limited accuracy. Future research is consequently required to validate the vitamin D FFQ for use in males and other populations.
- Sixth, subjects might have overestimated the vitamin D and calcium intake from the FFQ, limiting the accuracy of the vitamin D and calcium intake assessments.
- Seventh, 24-HDRs might given rise to some errors since it requires short term memory. Single observation in the 24-HDRs also limited the determination of the usual dietary consumption. A dietary record covering two or more days is advisable.
- Eighth, since some of the subjects failed to provide information about the brand name and dose of the supplements used, the present study did not include the amount of vitamin D received by the subjects daily; it was difficult to determine whether the supplement contained vitamin D2 or D3. As recommendation, the brand name and amount of vitamin D content of the supplements used by the subjects should be provided in detail in future studies.

- Ninth, since the assay used to determine vitamin D concentrations and the cut-off points of vitamin D vary between studies, it was difficult to compare the vitamin D status between studies.
- Tenth, the assay used in this study only measured vitamin D3 and was unable to measure 25(OH)D2 resulting in underestimation of vitamin D status. Future research using total vitamin D to measure vitamin D concentration is therefore recommended to ensure vitamin D status of our populations. However, previous literature noted that the assay was in good overall agreement as determined by LC-MS/MS and RIA (Leino, et al. 2005). In addition, the interferences in the assay caused by visible signs of hemolyzed sample resulting in falsely elevated results.
- Eleventh, limitation can be due to the cross-sectional nature of this study in which it was conducted at one specific time and gives no clue to the sequence of events.
- Twelfth, a different screening method was used to get information about osteoporosis between the urban and rural women. As suggestion, rural women should be provided with BMD scan.
- Thirteenth, the number of subjects recruited in urban area is approximately 3 times lesser than the rural subjects. In addition, there is imbalance proportion between different ethnicities. Recruiting more subjects in urban area, especially Malay women (since more Malay in rural area) are advisable.

The present study evaluated the vitamin D status of urban and rural women in Malaysia. The results showed the high prevalence of vitamin D deficiency and insufficiency especially in urban women. Increasing sun exposure, increased intake of dietary vitamin D and vitamin D supplements are necessary especially for those staying in urban areas.

The public health authorities and government agencies also should consider the fortification of food products with vitamin D.

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APPENDICES