Original Article

# Vitamin D status among postmenopausal Malaysian women

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Serum levels of 25-hydroxyvitamin D (25 (OH) D) were determined in 276 (103 Malays and 173 Chinese) postmenopausal women, aged 50 to 65 years. The level of 25 (OH) D was significantly lower in the postmenopausal Malay women (44.4 ± 10.6 nmol/L) compared to the Chinese women (68.8 ± 15.7 nmol/L) ($P$<0.05). There were 27% Malay women with serum 25 (OH) D in the range of 50 – 100 nmol/L (defined as lowered vitamin D status, or hypovitaminosis D) and 71% with levels in the range of 25 – 50 nmol/L (defined as vitamin D insufficiency) compared to 87% and 11% Chinese women respectively. Serum 25 (OH) D was found to significantly correlate with BMI, fat mass and PTH level. Multivariate analyses showed that race has a strong association with vitamin D status. The high prevalence of inadequate levels of serum vitamin D found in our study may have important public health consequences and warrants the development of a strategy to correct this problem in the older adult Malaysian population.
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**Key Words: 25 (OH) vitamin D, parathyroid hormone, vitamin D deficiency, diet, postmenopausal women, Asian, Malay, Chinese, Malaysia**

## Introduction

Vitamin D is of major importance for bone to facilitate the absorption of calcium (and to some extent phosphate) from the diet.[1] In its hormonal form, calcitriol (1,25 $(OH)_2$ $D_3$), has an effect on osteoclast development and synthesis of osteocalcin by osteoblasts. Serum 25-(OH)D is the best indicator of vitamin D status, and is contri-buted to by vitamin D produced by the skin and obtained from dietary intake.[2] Low serum vitamin D levels have often been reported in free-living elderly Caucasian women [3-5] and in Asian women such as those in Saudi Arabia[6] and India.[7]

The reasons for low levels of vitamin D in elderly women are possibly due to inadequate exposure to sunlight and/or poor diet. In addition, aging decreases the skin's capacity to produce vitamin D.[8] There is also a decrease in the hydroxylation of vitamin D and in the responsiveness of the intestinal mucosa to circulating vitamin D levels in elderly individuals.[2] Vitamin D defi-ciency leads to poor calcium absorption, high serum PTH concentrations and accelerated bone loss.[9] It has also been reported that deficiency can lead to loss of muscle strength[10] and an increased likelihood of falling, which in turn increases the risk of fracture.

So far, no study has assessed the vitamin D status of the population in Malaysia. Such a study is relevant in view of the increasing prevalence of osteoporosis in the region and the known role of melanin in inhibiting the dermal synthesis of vitamin D. Thus, the present study was conducted to assess the vitamin D status in a population of healthy postmenopausal women and to examine influencing factors.

## Methods

### *Subjects*

Subjects were recruited through advertisements, senior citizens clubs, residential areas and religious centers within a 50 km radius of Kuala Lumpur. Respondents were initially screened for eligibility using a question-naire. Chinese and Malay women aged between 50 and 65 years, who were more than 5 years post-menopausal, were eligible for the study. Subjects were excluded if

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**Table 1.** Demographic characteristics of subjects (*N*=274)

| | Malay (*N*=101) | Chinese (*N*=173) |
|---|---|---|
| Age | 60 ± 3 | 59 ± 3* |
| Years after menopause (yrs) | 11 ± 5 | 9 ± 4 |
| Age at menopause (years) | 49 ± 5 | 50 ± 4 |
| Reproductive (years) | 35 ± 5 | 36 ± 5 |
| Cause of menopause | | |
| natural | 78.2 % | 84.4 % |
| surgical | 21.8 % | 15.6 % |
| Household income status | | |
| low (< RM 2000) | 62.4 % | 43.9 % |
| middle (RM 2001-5000) | 22.8 % | 41.0 % |
| high (> RM 5000) | 14.9 % | 15.0 % |

*mean ± SD

they had a history of bone disease or medical conditions that affect bone metabolism, if they were taking medications that affect bone metabolism (eg. hormone replacement therapy, thiazide diuretics, glucocorticoids) or had other chronic illnesses (such as diabetes, kidney disease, heart disease, or cancer). The screening yielded 276 subjects (103 Malays and 173 Chinese) who were eligible and who provided informed consent to participate in the study.

***Measurements***

*Three-day food records*

Subjects were asked to record their food intake for three days (two weekdays and a weekend). The subjects were instructed to record all foods at the time of eating in local household measurements (i.e. bowls, cups, glasses, teaspoons and tablespoons). Food photographs and matchbox sizes were used to better quantify food portion sizes. Written instructions were also given to the subject. All completed records were then checked immediately by research personnel for clarity and understanding of portion sizes. Nutrient content was calculated based on the Malaysian food composition table. Vitamin D content in foods were obtained from the database in the Nutritionist IV ( First Databank Inc, USA) diet software.

***Anthropometry***

Subjects weight and height were also measured, in light clothing and without shoes, using a digital balance (SECA, Germany) with height attachment, to the nearest 100 g and 0.1 cm respectively. The body mass index was calculated as weight/height.$^2$ Lean body mass and body fat were measured by dual-energy x-ray absorptiometry (DEXA, Lunar DPX-L, Madison, WI, USA) with the analysis software version 3.1.

***Physical activity***

The Physical Activity Scale for the Elderly (PASE) questionnaire (New England Research Institute (NERI), Inc. MA, USA) was used to measure the physical activity level of the subjects. This questionnaire had been found to be an easy, reliable and valid instrument for the assessment of physical activity in a general population of non-institutionalized older persons.[11] The questionnaire comprised self-reported occupational, household and leisure activities over a one-week period. The PASE was administered by interview or completed by subjects themselves. PASE scores were then calculated from weights and frequency values for each of the different types of activities**,** based on the scoring instructions provided by the PASE Administration and Scoring Manual.[12]

***Biochemical analysis***

A fasting venous blood sample was collected from each subject between 0900 and 1000 hours at each visit from the month of October to March. Second-void urine samples were collected from all subjects between the hours of 0830 and 0900. Serum calcium, phosphate, magnesium and alkaline phosphatase as well as urinary calcium, sodium and creatinine were measured using the Dimension Clinical Chemistry System (Dade International Inc., Deerfield, IL, USA). Intact parathyroid hormone (hPTH 1-84) was measured by immunoradiometric assay (IRMA) using the DiaSorin N-tact PTH SP IRMA kit (DiaSorin Inc., MN, USA). The inter-assay CV for PTH measurement was 15%. A liquid phase radioimmunoassay kit (Gamma B, IDS Limited, USA) was used to extract and quantify 25(OH) D in serum. The inter-assay CV was between 7 to 9%.

***Statistical analysis***

Descriptive statistics were used to obtain the mean and standard deviation of the various parameters. Pearson's correlation and multiple linear regression were used to assess relationships between various indices in the subjects. Differences between groups were compared by t-test. All *P* values were two-tailed. The Statistical Package for Social Sciences (SPSS) software version 10.0 (SPSS Inc., Chicago, USA) was used for the data analyses. The study protocol was approved by the research ethics committee of the National University of Malaysia and University Malaya Medical Center. All subjects provided written informed consent.

**Results**

The characteristics of the subjects are shown in Table 1. The majority of the subjects were from the low to middle income group. The mean age of the subjects was 59 ± 3 years. The average age of menarche was 14 ± 3 years old and the average years since menopause was 9 ± 4 years with 82 % of them undergoing menopause naturally and another 18 % having surgically induced menopause. The average age attained at menopause was 49 ± 4 years old and the mean span of reproductive years was 35.9 ± 4.9 years. Table 2 shows the physical characteristics and biochemical profiles of the subjects. There was a significant difference in body weight between the Malay and Chinese women, with the Malays being heavier ($P<0.05$). A higher BMI was also observed in the Malays compared to the Chinese ($P<0.001$). The Malay subjects' mean values for serum calcium, alkaline phosphatase (ALP) and PTH were higher than those of the Chinese, but were not significantly different. However, the 25 (OH) D concentration for the Malays was significantly lower compared to the Chinese ($P<0.001$). It is also shown in Table 2 that Malay women were significantly ($P<0.05$) less active than Chinese women.

**Table 2.** Anthropometry, bone mineral density and biochemical characteristics of subjects ( mean ± SD)

| | Malays (*N*=101) | Chinese (*N*=173) |
|---|---|---|
| Anthropometry | | |
| Weight (kg) | 62.5 ± 11.1 | 56.8 ± 9.2* |
| Height (m) | 1.52 ± 0.05 | 1.54 ± 0.05 |
| BMI (kg/m$^2$) | 27.2 ± 4.9 | 23.8 ± 3.6** |
| Fat mass (kg) | 25.4 ± 7.6 | 20.2 ± 7.0* |
| Lean body mass (kg) | 33.5 ± 3.9 | 32.9 ± 3.9 |
| Biochemical | | |
| Serum calcium (mmol/l) | 2.31± 0.11 | 2.29 ± 0.09 |
| Alkaline phosphatase(IU/l) | 86 ± 25 | 83 ± 22 |
| PTH ( pmol/l) | 2.81 ± 1.59 | 1.93 ± 1.08 |
| 25-OH vitamin D (nmol/l) | 44.4 ± 10.6 | 68.8 ± 15.7** |
| Activity scores | 82.82 ± 45.10 | 106.23 ± 57.12* |

* significant difference between groups at $P<0.05$;
** significant difference between groups at $P<0.001$

**Table 3.** Nutrient intake per day of subjects (mean ± SD)

| | Malays (*N*=101) | Chinese (*N*=173) |
|---|---|---|
| Calories (kcal) | 1747 ± 483 | 1550 ± 294** |
| % carbohydrate | | 53 |
| % protein | 15 | 16 |
| % fat | 29 | 29 |
| Protein (g) | 67 ± 29 | 66 ± 16* |
| Carbohydrate (g) | 244 ± 74 | 206 ± 41* |
| Fat ( g) | 56 ± 19 | 52 ± 15* |
| Calcium ( mg) | 549 ± 301 | 468 ± 216** |
| Phosphorus (mg) | 1020 ± 381 | 853 ± 234** |
| Vitamin D (μg) | 9.1 ± 12 | 8.4 ± 19 |

The activity scores were 82.8 ± 45.1 for the Malays and 106.2 ± 57.1 for the Chinese. The majority of the Chinese (62.5%) reported that they exercised regularly. The main types of exercise performed were Tai chi, brisk walking and dancing, whereas less than half (46.5%) of the Malays reported exercising regularly.

Table 3 shows significantly higher mean values among the Malays for energy, protein, carbohydrate, fat, calcium and phosphorus intakes compared to the Chinese ($P<0.05$). The vitamin D intake of the subjects was not found to be significantly different between the two races. It was lower than the adequate daily intake of 10μg for women 51 - 70 yr of age set by the Food & Nutrition Board.[13] The kind of food consumed by both races are shown in Table 4.

The cut-off point to determine adequate serum concentrations of 25 (OH) D is still subject to debate.[1] However, a graduated scale has been proposed in which hypovitaminosis D is defined as a serum 25 (OH) vitamin D concentration between 50 - 100 nmol/L, vitamin D insufficiency as a 25 (OH) D concentration between 25 - 50 nmol/L and vitamin D deficiency as a 25 (OH) D concentration <25 nmol/L.[11] Using these cut-off points, Table 5 shows that 88% of the Chinese subjects were classified as having hypovitaminosis D and 12% of the subjects were vitamin D insufficient. A different situation was observed among the Malays where 27% had hypovitaminosis D and 71% were found to be vitamin D insufficient.

**Table 4.** Types of food consumed by subjects (mean ± SD)

| Type of Food | Malay g/day | Chinese g/day |
|---|---|---|
| Fish | 71.29 ± 38.79 | 66.89 ± 45.75 |
| Poultry | 60.79 ± 46.54 | 62.63 ± 39.73 |
| Mutton | 20.00 ± 14.14 | 15.56 ± 6.94 |
| Beef | 35.69 ± 24.72 | 26.11 ± 21.07 |
| Duck | - | 31.92 ± 11.12 |
| Pork | - | 36.32 ± 30.76 |
| Internal organs | 18.33 ± 5.77 | - |
| Milk & milk products | 41.98 ± 40.25 | 33.41 ± 31.37 |
| Egg & egg products | 19.21 ± 13.56 | 16.13 ± 10.22* |

*Significant difference at $P<0.05$

**Table 5.** Vitamin D status of subjects

| Serum 25(OH)D | Malays | Chinese |
|---|---|---|
| 50 - 100 nmol/l | 26.7 %, mean = 57.1± 8.0* | 87.8%, mean = 71.1 ± 12.6 |
| 25 – 50 nmol/l | 71.3%, mean = 39.9 ± 6.1 | 12.2%, mean = 44.7 ± 4.8 |
| Less than 25 nmol/l | 2.0%, mean = 21.5 ± 2.6 | 0.0% |

*mean ± SD values

Table 6 shows the correlation matrix of the various indices for the total population studied. Vitamin D status was negatively correlated with BMI, fat mass and PTH. When the two groups were studied separately, serum 25 (OH) D levels of the Malay subjects were shown to be inversely correlated with fat mass (r =-0.205, $P<0.05$) and PTH concentration (r =-0.205, $P<0.05$) (Figure 1 & 2). Further analysis with multiple linear regression showed that serum 25(OH)D was influenced by race (Table 7), which contributed significantly to vitamin D status ($r^2 = 0.426$ ).

**Discussion**

Serum 25 (OH) D was found to be lower amongst the Malays than the Chinese postmenopausal women. This could be explained by the differences in skin pigmentation, activity levels or fat mass between the two groups. Need *et al*.,[15] suggested that the inverse relationship between 25 (OH) D and BMI may be due to a larger body pool of vitamin D and 25 (OH)D or to slower saturation and mobilization of these compounds from adipose tissues or both. Obese people have decreased bioavailability of vitamin D from cutaneous and dietary sources because of a tendency for vitamin D to deposit in adipose tissue.[16] An alternative explanation was proposed by Bell *et al.,*[17] who indicated that the vitamin D endocrine system

**Table 6.** Correlations of anthropometric characteristics with serum 25 (OH) D levels

| Parameter | Pearson r | *P* value |
|---|---|---|
| Age | -0.05 | 0.428 |
| BMI | -0.27** | 0.000 |
| Fat mass | -0.24** | 0.000 |
| PTH | -0.30** | 0.000 |
| Activity scores | 0.12 | 0.004 |

**significant correlations at $P<0.001$

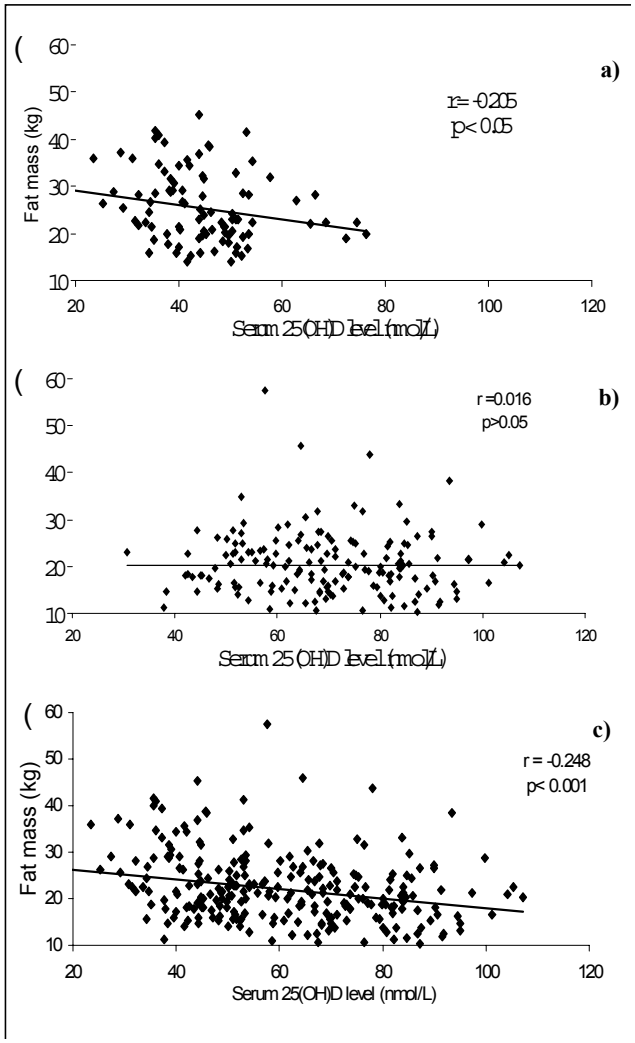


**Figure 1.** Relationships between serum 25(OH)D and fat mass for (a) Malay (b) Chinese (c) Total group of postmenopausal women

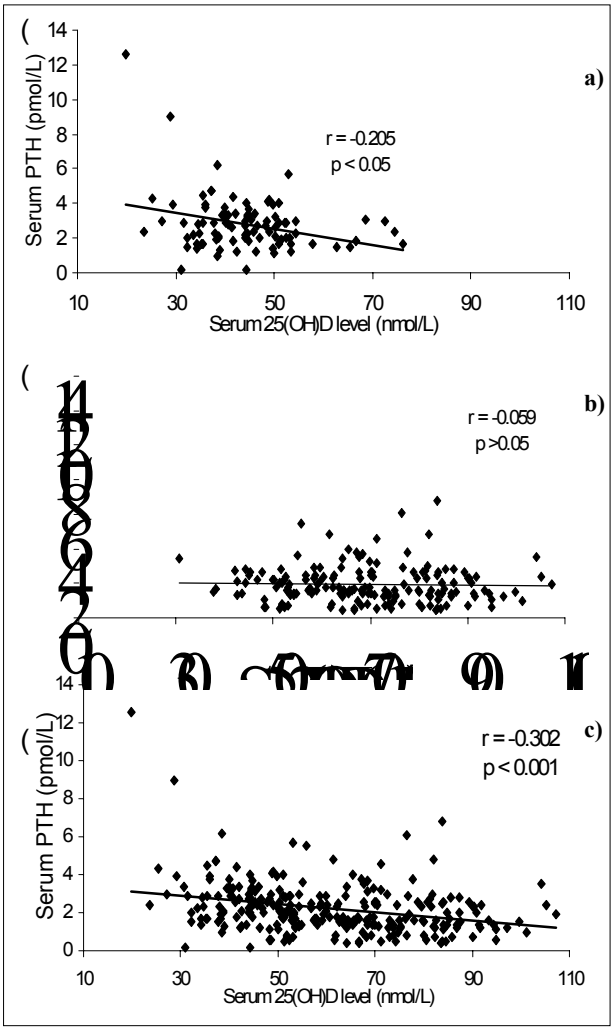


**Figure 2.** Relationships between 25-OH Vitamin D and PTH for (a) Malay (b) Chinese (c) Total group of postmenopausal women

**Table 7.** Multiple linear regression for serum 25 (OH) D with selected variables

| Variable | B | Std error | t | *P* |
|---|---|---|---|---|
| Race | 22.467 | 2.066 | 10.877 | 0.000** |
| Body fat mass (kg) | - 0.07382 | 0.121 | -0. 608 | 0.544 |
| Activity scores | 0.01884 | 0.016 | 1.146 | 0.253 |
| PTH | - 1.307 | 0.678 | - 1.928 | 0.055 |

**$P$<0.001

in obese people is altered, with increased production of 1,25 dihydroxyvitamin D exerting negative feedback control on hepatic synthesis. However, no relationship was found between BMI and physical activity.

It is well documented that vitamin D status is inversely related to parathyroid hormone (PTH) levels.[18] A similar inverse relationship was observed between PTH and 25 (OH) D (r =-0.205, $P$<0.05) for the Malay but not for the Chinese postmenopausal women. This may be because 25 (OH) D levels were lower in the Malays resulting in stimulation of PTH production. This inverse relationship is of particular interest because PTH is a potent bone-resorbing agent and a slight elevation in serum PTH will lead to increased bone turnover and accelerated bone loss.[19] This sort of bone loss predisposes to fragility fractures, which can be prevented by vitamin D and calcium supplementation.[20-21] The inverse correlations between PTH and serum 25 (OH) D in the Malays was driven by the six subjects who had serum vitamin D 30nmol/L or less. This contrasts the situation in the Chinese where there was only one subject close to 30nmol/L. The correlation in the Malays disappears when these six subject were removed. This warrants further study with bigger sample sizes.

Hypovitaminosis D is an endemic problem in the elderly.[22] The major causes of vitamin D deficiency are deprivation of sunlight, a consequential decline in the synthesis of cutaneous vitamin $D_3$ and decreased renal hydroxylation of 25(OH)D to its metabolically active form by the aging kidney. Untreated vitamin insuffi-

ciency can progress to bone loss and thus to an increased risk of fracture, but this is further compounded with aging. The threshold serum concentration of 25 (OH) D insufficiency below which bone loss is likely to occur, has been the subject of much research over the last few years. An early sign of vitamin D insufficiency is the secondary increase in serum parathyroid hormone, which may still be within the 'upper normal range'.[23] For healthy elderly who are living in the community, the desirable level of 100nmol 25(OH)D/L should be achieved by regular sunshine exposure together with the consumption of fortified milk and/or other foods.[14] The most important source of vitamin D is from synthesis of the vitamin by sunlight exposure in the skin.[24] Factors that affect cutaneous absorption include the use of sun-block, the level of sunlight exposure (e.g. time of day), clothing habits and skin pigmentation due to the presence of melanin concentration.[25] The Malay women may be at particular risk of vitamin D deficiency due to their higher melanin concentration compared to the Chinese.[26-27] Malay women were also observed to be less active outdoors and thus, exposure to sunlight would be lower than the Chinese women. Their lack of adequate sunlight exposure could be due to indoor confinement to avoid the extreme heat of the mid-day sun and their clothing habits which exposed to only some UV radiation on face and hands during their daily living life activities. Malaysian eat many kind of fish and frequent fish consumption is believed to help maintain adequate concentration of serum 25 (OH) D. However, this was not reflected in the results obtained. From the dietary record the Chinese consumed fish like spanish mackerel, silver pomfret, anchovies, fish ball and fish cakes while the Malays consumed fish like indian mackerel, black pomfret, hardtail pomfret, sardines and anchovies. The consumption of eggs as well as milk and milk products, which are also a source of vitamin D, was higher among the Malays, but was only significant ($P$<0.05) for the consumption of egg and its product.

Nevertheless, the finding that many Malaysian postmenopausal women had low vitamin D status is a cause for concern as Malaysia is a country with abundant sunlight and yet these postmenopausal women are at increased risk of osteoporosis from lack of vitamin D.

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