MAINTENANCE VITAMIN D3 DOSAGE REQUIREMENTS IN CHINESE WOMEN WITH POST MENOPAUSAL OSTEOPOROSIS LIVING IN THE TROPICS

Running title: Osteoporosis, Race & Vitamin D in the tropics

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Abstract:

Background: Vitamin D3(cholecalciferol) dose required to maintain sufficiency in non-Caucasian women with postmenopausal osteoporosis (PMO) in the tropics has not been well studied. Some guidelines mandate 800-1000 IU Vitamin D/day but the Endocrine Society (US) advocates 1500-2000 IU/day to maintain 25-hydroxyvitamin-D (25(OH)D) concentration at >75 nmol/L.

Objectives: We aimed to establish oral cholecalciferol dose required to maintain 25(OH)D concentration at >75 nmol/L in PMO Chinese Malaysian women, postulating lower dose requirements amongst light-skinned subjects in the tropics.

Subjects/Methods: 90 Chinese Malaysian PMO women in Kuala Lumpur, Malaysia (2°30’N) with baseline serum 25(OH)D levels ≥ 50 nmol/L were recruited. Prior Vitamin D supplements were discontinued and subjects randomized to oral cholecalciferol 25,000 IU/4-weekly (Group-A) or 50,000 IU/4-weekly (Group-B) for 16 weeks, administered under direct observation. Serum 25(OH)D, PTH, serum/urinary calcium were measured at baseline, 8 and 16 weeks.

Results: Baseline characteristics, including osteoporosis severity, sun exposure (~3 hours/week), and serum 25(OH)D did not differ between treatment arms. After 16 weeks, 91% of women sufficient at baseline, remained sufficient on 25,000 IU/4-weekly compared to 97% on 50,000 IU/4-weekly with mean serum 25(OH)D 108.1 ± 20.4 and 114.7 ± 18.4 nmol/L respectively (p=0.273). At trial’s end, 39% and 80% of insufficient women at baseline attained sufficiency in Group A and Group B (p=0.057). Neither dose was associated with hyperparathyroidism or toxicity.

Conclusions: Despite pretrial Vitamin D supplementation and adequate sun exposure, 25.6% Chinese Malaysian PMO women were Vitamin D insufficient indicating sunshine alone cannot ensure sufficiency in the tropics. Both ~900 IU/day and ~1800 IU/day cholecalciferol
can safely maintain Vitamin D sufficiency in >90% of Chinese Malaysian PMO women.

Higher doses are required with baseline concentration $<75\text{nmol/L}$.

**Keywords:**
- Vitamin D, oral cholecalciferol, postmenopausal osteoporosis, Chinese ethnicity, tropical

**Introduction**
Osteoporosis and its associated morbidity/mortality is a major public-health issue in the rapidly aging demographic of East and Southeast-Asia.\(^1\) Subjects of Chinese ethnicity are especially vulnerable to osteoporosis as corroborated by the fact that hip-fracture incidence in Malaysian women $>$age 50 years is highest amongst those of Chinese descent.\(^2\)

Vitamin D supplementation, coupled with anti-resorptive therapy and adequate calcium intake, form the cornerstones of postmenopausal osteoporosis (PMO) management.\(^3\) Vitamin-D plays an important role in calcium metabolism, bone health and muscle strength.\(^3\) Cholecalciferol supplementation reduces fracture risk and increases lower extremity strength, however these effects have been shown to be dose dependent. 80-90% of Vitamin-D is derived from cutaneous synthesis upon sun exposure, while 10-20% is obtained from dietary sources.\(^4\)

Hypovitaminosis D is a global problem affecting both northern and even more southern latitude populations. Vitamin D inadequacy ($<75\text{nmol/L}$) has been demonstrated in 52% of a large cohort of North American PMO women despite active osteoporosis treatment.\(^5\) In recent times, evidence of prevalent Vitamin D inadequacy has also emerged in sun rich areas such as Hawaii, Saudi Arabia and Southern India.\(^6,7\) Studies have also documented widespread Vitamin-D inadequacy in China\(^8\) and amongst South Korean/Japanese PMO women (some of whom were already Vitamin D supplemented).\(^9\)
Vitamin D inadequacy is also prevalent in tropical equatorial Malaysia, with evidence of ethnicity and sun exposure being important predictive factors. There have been only 4 published studies examining serum 25(OH)D levels amongst women in Malaysia and Singapore, two Southeast Asian countries with large immigrant Chinese populations and year-long sunshine. Only of one of these however studied women with confirmed PMO. Lips et al,\textsuperscript{10} reported a 0% prevalence rate of Vitamin D deficiency amongst a mixed population of women confirmed to have PMO - both Vitamin D supplemented and unsupplemented, living in Singapore (a majority-Chinese population). Amongst \textit{pre-menopausal} women who did not receive Vitamin-D supplementation in Kuala Lumpur, Green et al\textsuperscript{6} found a lower rate of Vitamin D deficiency amongst the Chinese (38%) compared with Malays (74%). Echoing these findings of an apparent ethnic ‘protection’ from Vitamin D deficiency amongst Chinese in a tropical setting, Rahman et al\textsuperscript{11} evaluating Vitamin D supplement naïve \textit{postmenopausal} women discovered a high prevalence of Vitamin D deficiency ($<$50nmol/L) (71%) amongst those of Malay ethnicity and ~6-fold lower prevalence (11%) amongst urban Chinese. It is not known however if these women had PMO as their BMD was not assessed. On the other hand, a Malaysian study by Musa et al,\textsuperscript{12} found that 81.3% of an urban population of perimenopausal Chinese women (52.3% total population) without osteoporosis, despite minimal sunscreen use had Vitamin D levels $<$50nmol/L compared with 11.9% of a rural population of predominantly Malay women (84%); with the main factor predictive of deficiency being reduced sun exposure amongst urban women.

Many osteoporosis guidelines\textsuperscript{13,14,15,16} mandate a minimum of 800-1000 IU/day Vitamin D as an essential component of osteoporosis therapy. There is less clarity when it comes to what optimal serum 25(OH)D concentration is and how much Vitamin D is required to maintain an optimal concentration. While it is well established that a high dose Vitamin D loading regimen is required as initial treatment of Vitamin-D deficiency
in order to attain a concentration of $>75\text{nmol/L}$, there has been little scientific inquiry into the dose of cholecalciferol required to maintain serum concentration of $>75\text{nmol/L}$ in non-Caucasian subjects living in the tropics, where sun exposure, cultural practices and skin pigmentation may differ. Neither is there agreement on the definition of Vitamin D sufficiency with some researchers targeting concentration of serum $25(\text{OH})\text{D}$ of $>50\text{nmol/L}$ and others aiming for $>75\text{nmol/L}$. The IOM (Institute of Medicine) 2010 guidelines, aiming for a lower serum $25(\text{OH})\text{D}$ target of $>50\text{nmol/L}$, advocate maintenance doses of 600IU/day in post-menopausal women aged 51-70 years and 800IU/day for those aged $>70$. These recommendations were made allowing for minimal sun exposure and were meant for healthy subjects, i.e. those without osteoporosis. In contrast, the Endocrine Society 2011 guidelines state that maintenance doses up to 1500-2000IU/day may be required to attain the higher optimal target of $>75\text{nmol/L}$ ‘in those at risk of vitamin D deficiency and falls/fractures based on high quality evidence’. This threshold serum Vitamin-D concentration of $75\text{nmol/L}$ is recommended based on the fact that secondary hyperparathyroidism is usually seen with concentration of $<75\text{nmol/L}$ (but not above), and that fractional absorption of calcium does not increase with Vitamin D supplementation in subjects with concentration of $>75\text{nmol/L}$. Similarly the 2010 Canadian guidelines, recommending a minimum of 800IU Vitamin-D/day for treatment of osteoporosis, have a caveat that $>1000\text{IU/day}$ may be required to attain optimal Vitamin D status. The 2013 UK National Osteoporosis Society Practical Guideline on the other hand, in accord with the IOM considers serum $25(\text{OH})\text{D}$ concentration above $50\text{nmol/L}$ as being within the optimal range and advocates treatment of those with osteoporosis (and Vitamin-D deficiency) on potent antiresorptives with a maintenance dose of 800-2000IU/day, making allowances for dark skinned populations and those with religious/cultural dress-codes limiting sun exposure.
Very few interventional studies focusing on the appropriate dose of Vitamin D required to maintain sufficiency have been conducted in East-Asian/Oriental subjects with PMO at high risk of fracture. Neither can results of studies in Japan, Korea and China\textsuperscript{19} be extrapolated to immigrant Chinese living in equatorial Southeast Asia where climate and diet differ. It is possible that Chinese women in Malaysia having more sun exposure than their northern Oriental counterparts and Caucasians may require lower supplementary Vitamin D doses to maintain adequacy. However, the effect of age on the skin’s ability to synthesize Vitamin D and cultural practices such as sun-avoidance may confound the aging PMO Chinese woman’s ability to maintain Serum 25(OH)D $>75\text{nmol/L}$.

Our study focuses on determining the maintenance dose of Vitamin D supplementation in women at high risk of fracture i.e. PMO Chinese women. Knowing that a minimum of 800 IU of Vitamin-D\textsuperscript{17,20,21} can prevent both falls/fractures regardless of Vitamin D status from studies in Caucasian populations of postmenopausal women and that 700-1000 IU/day may maintain concentration of $>75\text{nmol/L}$ in 50% of the Caucasian population,\textsuperscript{21} we therefore designed a prospective randomized controlled trial comparing the ability of a low(~900IU/daily) and high(~1800IU/daily) maintenance dose of oral cholecalciferol (Vitamin D3) to sustain serum concentration of $>75\text{nmol/L}$ amongst community dwelling Chinese women with PMO living in Kuala Lumpur, Malaysia which is located 2° 30’N, postulating that a lower dose of ~900IU daily would be required amongst this light skinned ethnic group living in a tropical climate with yearlong sunshine.

**Subjects and Methods**

A total of 142 community dwelling Chinese Malaysian women with postmenopausal osteoporosis, over the age of 55, attending University of Malaya Medical Centre Osteoporosis Clinic in Kuala Lumpur, Malaysia(2°30’ N) with baseline serum Vitamin D concentration of
were invited to participate in the study. 90 women who met the inclusion criteria were recruited. (Figure 1). We excluded patients with: malabsorption (prior history of colectomy, Roux-en-Y gastric-bypass), known metabolic disease other than PMO and secondary osteoporosis (granulomatous diseases, thyrotoxicosis, glucocorticoid-induced osteoporosis, liver/renal disease). Patients on over-the-counter Vitamin D supplements and medications that affect Vitamin D metabolism (rifampicin, estrogen, glucocorticoids, anticonvulsants) were also excluded. Participants agreed to refrain from using sunscreen, altering dietary calcium intake and sun exposure or from taking self prescribed calcium or Vitamin D supplements during the study. This study was approved by an institutional review board and signed informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

Subjects had a baseline screening visit with serum 25(OH)D level measurement, followed by an initial randomization visit and then visits at 4, 8, 12 and 16 weeks from randomization. Baseline laboratory tests included renal and liver function tests and serum calcium, phosphorus, 25-(OH)D, intact PTH, and albumin levels with 24 hour urinary calcium measurements. All relevant data were obtained via an interview, physical examination and questionnaire as well as from medical records. Patients were administered a validated sun exposure questionnaire²² and degree of skin pigmentation was quantified using Von Luschan’s Skin Colour Chart.²³ Study subjects’ baseline characteristics are summarized in Table 1.

At the second visit, eligible patients with serum Vitamin D concentration of >50nmol/L had their previous Vitamin D supplements discontinued and were block randomized to either receive 50,000 IU oral cholecalciferol (Vitamin D3) or 25,000 IU every 4 weeks for 16 weeks. Serum 25(OH)D levels, serum calcium, phosphorus, serum intact PTH, serum albumin levels and 24 hour urinary calcium measurements were evaluated once again.
at 8 and 16 weeks post-randomization. Any concomitant medications or adverse events were recorded at 4-weekly visits.

Oral cholecalciferol was ingested monthly under direct supervision during study visits. Each dose of cholecalciferol was diluted in 200ml of water. The cholecalciferol used in this study was formulated as spray dried powder stabilized with DL-alpha tocopherol (dry Vitamin D3 100 SD/S) (DSM Nutritional Products Switzerland Ltd). All subjects were also assigned to receive 1g of calcium carbonate daily.

**Definitions**

Vitamin D deficiency is defined as serum 25(OH)D concentration of <50nmol/L, insufficiency as serum 25(OH)D of 50-<75nmol/L, sufficiency as 75-250nmol/L and Vitamin D intoxication as serum 25(OH)D concentration exceeding 250nmol/L. Secondary hyperparathyroidism is defined as normal serum calcium with a PTH above upper limit of normal (>6.8pmol/L).

Osteoporosis is defined as one of the following: bone mineral density (BMD) T score ≤-2.5 at any site or written documentation of diagnosed osteoporosis in medical chart or low trauma, non-pathological fragility fracture of the hip, spine, wrist, humerus or clavicle after age 45, on current or previous treatment for osteoporosis with any approved osteoporosis medication. Prior to study enrolment, patients had their bone mineral densities evaluated by DXA [DPX IQ 240 GE device/Lunar Prodigy device (GE Medical Systems Lunar, Madison, WI, USA)].

**Biochemistry**

Serum 25(OH)D concentration was measured by an electro-chemiluminescence immunoassay (ECLIA) from Roche Diagnostics (minimum detectable concentration:
7.5nmol/L, maximum: 175.0nmol/L) Intra assay precision (coefficient of variation): mean of 38.4nmol/L- 6.9%, mean of 169.5nmol/L- 1.7%. Inter assay variability: mean of 38.8 nmol/L- 12.2%, mean of 169.5nmol/L -2.2%.

Serum PTH was measured by the Elecsys analyzer using ECLIA for quantitative determination of intact PTH in serum (minimum detectable level: 0.1pmol/L, maximum: 52.5pmol/L). Intra-assay precision: mean of 2.1pmol/L - 4.1%, mean of 6.1pmol/L - 2.2%. Inter-assay variability: mean of 2.1pmol/L - 6.2%, mean of 6.1pmol/L - 4.1%. The reference range for serum PTH is 1.6-6.8pmol/L.

Serum and urinary calcium were measured by O-cresolphthalein complex using automated equipment [Dimension Vista (Siemens)].

**Statistical Analysis**

Statistical Analysis was performed using SPSS 16.0J for Windows. Descriptive statistics are reported as mean ± SD. Baseline comparisons were analyzed using Student’s t test or Mann–Whitney test depending on normality. A one-way between groups, analysis of covariance (ANCOVA) was conducted to compare effects of oral cholecalciferol supplementation. p value < 0.05 was considered statistically significant.

**Results**

**Baseline characteristics**

90 community dwelling Malaysian women of Chinese ethnicity with post menopausal osteoporosis were enrolled in this study. 82.2% of women were already on some form of Vitamin D supplementation (dose 200-1200IU/day) prior to study enrolment which was ceased on recruitment. At baseline, 42.2% of subjects were on 400 IU daily and 31.1% on ≥800 IU daily. 80% of the sample population were on active treatment for osteoporosis with
either alendronate or strontium. 45 subjects each were randomized to receive either 25,000 IU (Group-A) or 50,000 IU (Group-B) oral cholecalciferol every 4 weeks respectively. No significant differences were present at baseline between the 2 groups with regards to age, BMI, duration of menopause, severity of osteoporosis, sun exposure, skin colour, mean serum 25(OH)D, serum calcium, and 24 hour urinary calcium levels (Table 2). At baseline, 25.6% of the total 90 subjects had 25(OH) D concentration between 50.1-74.9 nmol/L (insufficiency) (n=23) and 74.4% had concentration of ≥75 nmol/L (sufficiency). At baseline, 82.6% and 82.1% of insufficient and sufficient subjects respectively had received Vitamin D supplementation prior to study enrolment. Median Vitamin D dose was significantly lower in the insufficient group at baseline compared with the sufficient group, 13% vs 37.3% of subjects were on ≥800 IU/day prior to study enrolment in the insufficient and sufficient at baseline subgroups respectively. There was no significant difference in sun exposure between the insufficient and sufficient subgroups at baseline with both spending a median of 2.5 and 2.25 hours/week in the sun respectively. (Table 1) Neither were there significant differences in age, BMI and sun exposure index between the insufficient and sufficient subgroups (data not shown). Mean concentration of Serum 25(OH) D in both the sufficient and insufficient subcategories did not differ significantly in both treatment arms (Table 3).

**Vitamin D status after treatment with monthly cholecalciferol**

Mean serum 25(OH)D concentration rose significantly from baseline to 16 weeks in both treatment groups, Group A: 90.2 ± 23.1 to 96.0 ± 24.1 SD nmol/L, Group B 91.6 ± 24.6 to 107.1 ± 22.7 SD nmol/L (Figure 2). After 16 weeks of oral cholecalciferol supplementation, in women who were already sufficient at baseline, 91% remained sufficient on 25,000 IU 4-weekly (low-dose therapy) while 97% of those on 50,000 IU 4-weekly (high-dose therapy) remained sufficient. These differences were not clinically significant. The drop
in Vitamin-D sufficiency rate from 100% at baseline, to 91% and 97% in Group A and B respectively after 4 months was also not clinically significant (p=0.273). Only 39% (5/13 women) and 80% (8/10 women) of those who were insufficient at baseline however attained sufficiency in Group A (low dose) and Group B (high dose) respectively - these differences however only approached clinical significance (p=0.057).

In the sample population as a whole, there were no significant differences in mean serum 25(OH)D concentrations between the low dose and high dose therapy groups at 8 weeks however at 16 weeks the mean vitamin D concentration in patients taking high dose monthly vitamin D was significantly higher(p=0.027). Amongst women with insufficient Vitamin D concentration at the start of the trial[Serum 25(OH)D level :50 - <75nmol/L], treatment with low-dose Vitamin D (Group-A) produced a significant increase in serum 25(OH)D concentration only after 16 weeks therapy, whereas treatment with high dose (Group B) produced significant increments at both 8 and 16 weeks. In those who were sufficient at baseline, low dose therapy produced no significant change in serum 25(OH)D at 8 or 16 weeks compared with baseline but high dose cholecalciferol produced significant increases at both time points.

**PTH suppression**

All patients were normocalcemic at baseline and end of study. At baseline, 15.6% and 8.9% of participants in the low dose (Group-A) and high dose (Group B) arms respectively had secondary hyperparathyroidism. All participants with secondary hyperparathyroidism attained serum PTH values within the normal range after 4 months treatment. Mean PTH levels were not significantly different between the Vitamin D insufficient and sufficient at baseline subgroups respectively (4.9 ±2.1 vs 4.5±1.7 pmol/L, p=NS). PTH levels declined
significantly in both treatment arms with Vitamin D therapy (Figure 3). There was no
significant difference in mean PTH levels between both treatment arms at any time point.

**Safety**

None of the subjects developed hypercalcemia or hypercalciuria during the course of
this study. There were no significant differences in corrected serum calcium or urinary
calcium excretion between treatment groups.

**Discussion**

Unexpectedly, a quarter of our cohort of Chinese-Malaysian women with PMO had
evidence of Vitamin D insufficiency (50 - <75nmol/L) at baseline despite the fact ~80% were
Vitamin D supplemented. Conversely only a small proportion of those sufficient at baseline
(17.9%) were not cholecalciferol-supplemented prior to study enrolment. In comparison with
women who were sufficient at baseline, the insufficient subgroup was on significantly lower
doses of Vitamin D i.e. only 13% were on ≥800IU/day compared with the sufficient
subgroup, 37.1% of whom were on ≥ 800 IU/day. Those who were insufficient at baseline
also had a median of 2.5 hours of sun-exposure/week which was not significantly different
from those who were sufficient at baseline. These findings indicate that sun exposure and diet
alone are insufficient to maintain adequate Vitamin D concentration in this high fracture risk
patient cohort of Chinese ethnicity. Ethical concerns precluded a placebo arm in our study,
given the fact that many guidelines advocate a minimum Vitamin-D dose of 800IU/day as one
of the cornerstones of osteoporosis therapy. There are several reasons
substantiating universal Vitamin D supplementation in the elderly PMO woman: 1) Vitamin D
therapy prevents falls and fractures in the elderly regardless of Vitamin D status. 2) Most
RCTs demonstrating efficacy of active osteoporosis therapy have done so in combination with
calcium and Vitamin D supplementation. 3) The assumption (evidenced by our cohort) that Vitamin D inadequacy is prevalent in the elderly. Indeed the fact that a significant proportion of our cohort were insufficient at baseline despite Vitamin D supplementation indicates that it is not a question of whether Vitamin-D supplementation is required but rather how much Vitamin D is necessary for optimal bone health amongst the Chinese diaspora to Southeast Asia.

We also found that a maintenance dose of 25,000 IU cholecalciferol (Vitamin D3) monthly for 16 weeks which is equivalent to ~900IU/day, is as efficacious as a dose of 50,000 IU monthly (~1800IU/day) in maintaining serum 25(OH) D concentration above 75nmol/L (Vitamin D sufficiency) in the majority of Chinese Malaysian women (>90%) with postmenopausal osteoporosis (PMO) who are already sufficient at baseline with no significant differences in PTH suppression. 16 weeks on the low dose regimen however cannot normalize Vitamin D concentration in Chinese Malaysian PMO women with serum 25(OH)D concentration between 50-<75 nmol/L (Vitamin D insufficiency) despite adequate sun exposure of 2.5 hours/week. In the total study population, although mean serum 25(OH)D after 16 weeks therapy was significantly higher in patients enrolled in the high dose arm, both dosing regimens however resulted in average serum 25(OH)D concentrations either within or marginally above the 90-100 nmol/L range which has been associated with optimal bone health outcomes such as fracture reduction, improved BMD and improved lower extremity function. Importantly after 16 weeks therapy there were no patients with secondary hyperparathyroidism in both the low dose and high dose arms. Both the low dose and high dose maintenance regimes were safe with no cases of hypercalciuria, hypercalcemia or Vitamin D intoxication in either arm.

To our knowledge, this is the first published study comparing low dose and high dose Vitamin D supplementation amongst Chinese immigrants to tropical Southeast Asia. These
findings indicate that 900IU/day of Vitamin D may be sufficient to maintain sufficiency in most Chinese PMO women living in equatorial Malaysia who already have serum 25 (OH) D concentration of >75nmol/L. Based on good evidence in Western populations that the threshold levels for fracture risk reduction and fall prevention are 400 IU and 700-1000IU respectively, a maintenance dose of 900 IU daily may be presumed to not only maintain sufficiency in Chinese Malaysian women but also improve bone-health related clinical endpoints. It is important to remember however that our study participants were prohibited from using sunscreen. It is therefore possible that Chinese Malaysian women who use sun block may require doses higher than 900IU/day to maintain sufficiency.

Women with Vitamin D insufficiency (50 - <75nmol/L) at baseline however may require doses as high as 1800 IU daily or a high dose loading regimen similar to that advocated by the Endocrine Society for treatment of Vitamin-D deficiency i.e. 50,000 IU weekly for 8 weeks, followed by a low dose maintenance regimen of 900IU daily. Only 39% of women enrolled who were insufficient at baseline, attained sufficiency (>75nmol/L) after 16 weeks of low dose therapy compared with 80% in the high-dose treatment group. These differences in rates of attaining Vitamin D sufficiency approached clinical significance (p=0.057) and might have attained statistical significance if the sample size had been larger. It is possible, however, that a longer period of treatment with low dose Vitamin-D3 900IU/day extending up to perhaps 6 months may have been able to attain 25 (OH)D concentration exceeding 30 ng/ml in these women given that serum 25(OH)D continued to climb even in the latter half of this 16-week trial.

Others have also found that baseline serum 25(OH) D impacts on post treatment Vitamin D concentrations and that lower maintenance doses can suffice in those with higher baseline levels. A review by Bischoff-Ferrari et al quotes evidence that optimal fracture prevention occurs in trials with mean achieved levels of ~100nmol/L which were attained in
subjects where 700-800 IU/day was administered to subjects with mean baseline concentrations between 43.9-76.9 nmol/L. Trials in elderly postmenopausal Caucasian women living in Switzerland and Boston, USA in long-stay geriatric care/nursing home facilities have shown that while vitamin D doses of 800IU daily ± calcium can reduce falls, these patients who started with baseline concentrations <50nmol/L were unable to achieve mean Sr.25(OH)D >75nmol/L after 12 weeks and 5 months treatment under trial conditions respectively. On the other hand Chapuy et al, in their landmark RCT of 3270 healthy ambulant community-dwelling elderly postmenopausal women (mean age: 84 years) which demonstrated Vitamin D supplementation (+ calcium) reduced fractures, found that in a subgroup of 142 subjects in whom serum Vitamin D was measured, 800IU daily increased Sr.25(OH)D from a baseline <50nmol/L to levels of 100nmol/L at 6 months, maintaining concentrations at 105 nmol/L at 12 and 18 months respectively. These conflicting results of randomized placebo controlled trials in older Caucasian women living in northern latitudes seem to indicate that 800IU/day is an adequate dose to maintain Vitamin D sufficiency in ambulant subjects who may have the added benefit of sun-exposure, but is not efficacious in institutionalized subjects.

Baseline Vitamin D is also known to be influenced by latitude/sun-exposure amongst other factors, therefore it is entirely plausible that our cohort of light skinned Chinese PMO women living close to the equator with baseline Vitamin D concentration of >75nmol/L, require a lower maintenance dose of Vitamin D. The strength of our study design is that patient compliance was assured, as all Vitamin D was administered in hospital under direct observation. Our study is limited by the lack of detailed diet history with regards to fish, mushroom and egg intake (important natural sources of dietary Vitamin D). However, as Vitamin D fortified foods and fatty fish such as sardines, tuna and salmon are not widely available or consumed in Malaysia, this data may not have added much further information. It
may also have been beneficial to assess the efficacy and safety of the two different maintenance doses over a longer time-frame of 6-12 months instead of 4 months.

These findings indicate that Vitamin D therapy should be tailored to ethnic, geographical and cultural differences given the impact of these factors upon sun exposure and diet. It is not surprising that light skinned Chinese women in tropical Malaysia with year-long abundant sun exposure require lower doses of Vitamin-D compared with Caucasians living in northern latitudes subject to seasonal variation in UVB radiation. However, given the fact that dermal production of Vitamin-D upon sun exposure is compromised in the elderly\textsuperscript{4}, these results were not a foregone conclusion. In addition, Asian culture has a propensity to prize fair complexioned women and this may thus have necessitated higher doses of supplemental Vitamin D secondary to sun avoidance behaviour in our study population. Conversely, other studies seem to indicate that dark skinned Asian Indians and African Americans require higher than normal doses of Vitamin D to correct Vitamin D deficiency because of reduced UVB radiation penetration secondary to increased melanin pigmentation.\textsuperscript{26,27} Our findings indicate that that the 2011 Endocrine Society recommendations that 1500-2000 IU Vitamin D3/day may be necessary to raise serum 25(OH)D concentration above $75\text{nmol/L}$\textsuperscript{17} may not be applicable in Chinese Malaysian women who are already Vitamin D sufficient. Importantly, these results indicate that just as higher cholecalciferol doses are recommended in patients with obesity and those on anticonvulsants\textsuperscript{17} perhaps lower maintenance doses can suffice in light skinned individuals living in the tropics.

In conclusion, despite pre-trial Vitamin D supplementation and adequate sun exposure, 25.6\% of Chinese Malaysian PMO women are Vitamin D insufficient; indicating a need for cholecalciferol dose titration studies in this high fracture risk population. Our findings that both ~900IU/day and ~1800IU/day Vitamin-D3 can safely maintain serum 25(OH) D above $75\text{nmol/L}$ and suppress PTH in >90\% of Chinese Malaysian women with postmenopausal
osteoporosis who have Vitamin D concentration exceeding 75nmol/L at baseline, have
important public health consequences in Southeast Asia. These results highlight the need for
location specific dosing studies amongst different ethnic groups in this era of patient centered
treatment algorithms. Further studies examining long-term effects Vitamin D dose on falls
and fractures in Chinese immigrants living in Southeast Asia are warranted.

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Ethical Approval
The design of this study was approved by the institutional review board and local ethical
committee of the University of Malaya. Written and signed consent was obtained from all
patients in accordance with the Declaration of Helsinki.
References


Figure Legends

Figure 1 – Patient Flow Diagramme

Figure 2. Effect of monthly Cholecalciferol supplementation on serum 25(OH)D. After 16 weeks of supplementation, serum 25(OH)D increased to a greater extent from baseline with 50,000 IU/month compared to 25,000 IU/month with a mean level of 107.1 ± 22.7 nmol/L from a level of 91.6 ± 24.6 nmol/L in patients receiving 50,000 IU monthly oral cholecalciferol with an increment of 15.6 ± 16.3 nmol/L (p=<0.001) compared to a mean 25(OH)D level of 96.0 ± 24.1 nmol/L from a baseline level of 90.2± 23.1 nmol/L with an increment of 5.8 ± 16.0 nmol/L (p=0.019) in patients taking 25,000 oral cholecalciferol monthly.

Figure 3. Effect of monthly Cholecalciferol supplementation on serum PTH levels. No significant difference between the level of PTH supression in any groups at any time point. The mean PTH level at 6 months is less than baseline PTH levels after 16 weeks of supplementation. Serum PTH decreased by 0.5 ± 2.4 pmol/L (p=0.154) to reach mean value of 4.1 ± 1.7 pmol/L from a baseline value of 4.7 ± 1.8 pmol/L in subjects receiving 25,000 IU Cholecalciferol monthly. Subjects receiving 50,000 IU Cholecalciferol had a decline in PTH levels of 0.4 ± 1.6 pmol/L (p=0.108) from a baseline level of 4.6 ± 1.8 pmol/L to reach the level of 4.2 ± 1.2 pmol/L.
Table 1 – Baseline Characteristics*

<table>
<thead>
<tr>
<th>Baseline Parameters</th>
<th>Total Vitamin D Concentration</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;30 ng/mL, n=23</td>
<td>≥30 ng/mL, n=67</td>
</tr>
<tr>
<td>Age, years</td>
<td>Mean±SD 66.52±6.41</td>
<td>68.09±5.52</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>Mean±SD 22.73±2.76</td>
<td>22.77±3.84</td>
</tr>
<tr>
<td>Vitamin dose at baseline, IU</td>
<td>Mean±SD 360.87±325.78</td>
<td>538.51±375.32</td>
</tr>
<tr>
<td>PTH, pmol/L</td>
<td>Mean±SD 4.90±2.12</td>
<td>4.53±1.67</td>
</tr>
<tr>
<td>Fraction of BSA exposed, %</td>
<td>Median (IQR) 0.37 (0.21-0.45)</td>
<td>0.39 (0.26-0.45)</td>
</tr>
<tr>
<td>Hours of sun exposure per week, hours</td>
<td>Median (IQR) 2.50 (1.25-4.33)</td>
<td>2.25 (1.17-4.50)</td>
</tr>
<tr>
<td>Skin Colour</td>
<td>Median (IQR) 26.00 (24.00-26.00)</td>
<td>26.00 (24.00-26.00)</td>
</tr>
<tr>
<td>Sun Exposure Index (SEI) #</td>
<td>Median (IQR) 0.93 (0.35-1.95)</td>
<td>0.79 (0.37-1.58)</td>
</tr>
</tbody>
</table>

*Data is expressed as mean ± SD and median (Interquartile range) depending on normality. Reference laboratory ranges are as follows: 25(OH)D, 3–70 ng/ml; iPTH, 1.6–6.8 pmol/L; Serum Ca, 2.12mmol/L – 2.52mmol/L; 24 hour urinary Ca, 1.0 – 8.8 mmol/ 24 hours

#Sun Exposure Index (SEI) = Mean Body Surface Area x Total Exposure Hours per Week
<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Group A Patients receiving 25,000 IU Vitamin D monthly (n=45)</th>
<th>Group B Patients receiving 50,000 IU Vitamin D monthly (n=45)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>68 ± 5</td>
<td>67 ± 6</td>
<td>0.586</td>
</tr>
<tr>
<td>Years after menopause</td>
<td>17 ± 8</td>
<td>17 ± 7</td>
<td>0.937</td>
</tr>
<tr>
<td>Age at menopause (years)</td>
<td>51 ± 4</td>
<td>50 ± 4</td>
<td>0.560</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>152.8 ± 4.8</td>
<td>153.4 ± 5.7</td>
<td>0.587</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>53.5 ± 8.9</td>
<td>53.2 ± 9.0</td>
<td>0.867</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.9 ± 3.7</td>
<td>22.6 ± 3.4</td>
<td>0.651</td>
</tr>
<tr>
<td>Vitamin D intake &lt; 400 IU/day</td>
<td>10 (22.2)</td>
<td>13 (28.9)</td>
<td>0.733</td>
</tr>
<tr>
<td>Vitamin D intake ≥ 400 - &lt; 800 IU/day</td>
<td>21 (46.7)</td>
<td>18 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Vitamin D intake ≥ 800 IU/day</td>
<td>14 (31.1)</td>
<td>14 (31.1)</td>
<td></td>
</tr>
<tr>
<td>BMD spine (g/cm²)</td>
<td>0.823 ± 0.116</td>
<td>0.848 ± 0.135</td>
<td>0.348</td>
</tr>
<tr>
<td>T score spine</td>
<td>-2.7 ± 0.8</td>
<td>-2.5 ± 0.9</td>
<td>0.338</td>
</tr>
<tr>
<td>BMD neck of femur (g/cm²)</td>
<td>0.697 ± 0.099</td>
<td>0.694 ± 0.104</td>
<td>0.880</td>
</tr>
<tr>
<td>T score neck of femur</td>
<td>-2.1 ± 0.8</td>
<td>-2.2 ± 0.8</td>
<td>0.364</td>
</tr>
<tr>
<td>BMD total hip (g/cm²)</td>
<td>0.803 ± 0.139</td>
<td>0.763 ± 0.096</td>
<td>0.115</td>
</tr>
<tr>
<td>T score total hip</td>
<td>-1.8 ± 1.1</td>
<td>-2.0 ± 0.8</td>
<td>0.160</td>
</tr>
<tr>
<td>Patients with Falls</td>
<td>16 (35.6)</td>
<td>23 (51.1)</td>
<td>0.136</td>
</tr>
<tr>
<td>Patients with Previous Fractures</td>
<td>16 (35.6)</td>
<td>23 (51.1)</td>
<td></td>
</tr>
<tr>
<td>Serum Corrected Calcium (mmol/L)</td>
<td>2.26 ± 0.10</td>
<td>2.30 ± 0.13</td>
<td>0.142</td>
</tr>
<tr>
<td>Serum Albumin (g/L)</td>
<td>40.9 ± 2.7</td>
<td>40.8 ± 2.5</td>
<td>0.841</td>
</tr>
<tr>
<td>Baseline 25(OH) D level (nmol/L)</td>
<td>90.2 ± 23.1</td>
<td>91.2 ± 24.6</td>
<td>0.788</td>
</tr>
<tr>
<td>PTH level (pmol/L)</td>
<td>4.7 ± 1.8</td>
<td>4.6 ± 1.8</td>
<td>0.856</td>
</tr>
<tr>
<td>24 hour urine calcium (mmol/24 hours)</td>
<td>4.26 ± 2.40</td>
<td>3.66 ± 2.31</td>
<td>0.230</td>
</tr>
<tr>
<td>Skin Colour #</td>
<td>25.1 ± 1.9</td>
<td>24.6 ± 2.0</td>
<td>0.201</td>
</tr>
<tr>
<td>Sun Exposure Index **</td>
<td>1.22 ± 1.32</td>
<td>1.15 ± 1.13</td>
<td>0.803</td>
</tr>
<tr>
<td>Mean Sun Exposure (Total Hours/week)</td>
<td>3.00 ± 2.91</td>
<td>3.20 ± 2.64</td>
<td>0.728</td>
</tr>
<tr>
<td>Mean Body Surface Area (m²)</td>
<td>0.36 ± 0.13</td>
<td>0.34 ± 0.11</td>
<td>0.423</td>
</tr>
</tbody>
</table>

Table 2 – Baseline Characteristics After Randomization

*Reference laboratory ranges are as follows: 25(OH)D, 7.5–175.0 nmol/L; iPTH, 1.6–6.8 pmol/L; Serum Ca, 2.12–2.52 mmol/L; 24 hour urinary Ca, 1.0 – 8.8 mmol/24 hours

# Measured using Von Lushchan Skin Colour Chart

**Sun Exposure Index (SEI) = Mean Body Surface Area x Mean Sun Exposure

Data is expressed as mean ± SD. Comparison of indices between subjects in group (A) and group (B) were done with the Student’s t test or Mann–Whitney test depending on normality. Figures in brackets represent percentage of population in each group.
### Table 3 – Change in Vitamin D Concentration from Baseline after oral cholecalciferol supplementation

<table>
<thead>
<tr>
<th>Monthly Vitamin D dosage</th>
<th>Mean 25-OH D Concentration (nmol/L)</th>
<th>Mean 25-OH D Concentration (nmol/L)</th>
<th>Mean 25-OH D concentration (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients (Vitamin D ≥50nmol/L)</td>
<td>Insufficient at Baseline (50 - &lt;75nmol/L)</td>
<td>Sufficient at Baseline (≥75nmol/L)</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>8 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>16 weeks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects achieving Vitamin D sufficiency (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gp A 25,000 IU/mth</td>
<td>90.2 ± 23.1</td>
<td>62.6 ± 7.6</td>
<td>101.4 ± 17.0</td>
</tr>
<tr>
<td></td>
<td>91.5 ± 23.5</td>
<td>66.6 ± 10.8</td>
<td>101.6 ± 19.3</td>
</tr>
<tr>
<td></td>
<td>96.0 ± 24.1</td>
<td>72.3 ± 13.7</td>
<td>105.6 ± 20.4</td>
</tr>
<tr>
<td>Gp B 50,000 IU/mth</td>
<td>91.6 ± 24.6</td>
<td>107.1 ± 22.7</td>
<td>100.6 ± 20.0</td>
</tr>
<tr>
<td></td>
<td>97.7 ± 21.6*</td>
<td>80.4 ± 14.8</td>
<td>105.0 ± 18.2*</td>
</tr>
<tr>
<td></td>
<td>100.2 ± 21.7*</td>
<td>80.4 ± 14.8</td>
<td>114.7 ± 18.4*</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SD

* *p<0.05 (significant change in serum 25(OH)D concentration compared to baseline)

No statistically significant difference in changes in serum 25(OH)D concentration between Group A and B.
Figure 1 – Patient Flow Diagramme

142 post menopausal Chinese women over the age of 55 were invited to participate

52 excluded - did not meet inclusion criteria

90 subjects randomized to participate in interventional study

45 randomized to receive monthly oral cholecalciferol 50,000 IU

All subjects completed study with no dropouts

45 randomized to receive monthly oral cholecalciferol 25,000 IU

All subjects completed study with no dropouts
Figure 2 - Serum 25-hydroxyvitamin D concentration for all groups at all study time points

![Graph showing serum 25-hydroxyvitamin D levels across different time points and dosages.]

Figure 3 - Effect of monthly Cholecalciferol supplementation on serum PTH level.

![Graph showing the effect of different dosages of vitamin D3 on serum PTH levels over time.]

- Baseline
- 8 weeks
- 16 weeks

- Mean serum 25-hydroxyvitamin D level (nmol/L)
- Monthly Vitamin D3 Dosage:
  - 25,000 IU/mth
  - 50,000 IU/mth

- Mean PTH level (pmol/L)
- Monthly Vitamin D3 Dosage:
  - 25,000 IU/mth
  - 50,000 IU/mth