The role of ultraviolet-B from sun exposure on vitamin D3 and parathyroid hormone level in elderly women in Indonesia

ORIGINAL ARTICLE

S Setiati[1] MD, PhD, **M Oemardi**[2] MD, **B Sutrisna**[3] MD, PhD, **Supartondo**[4] MD

ABSTRACT

Objectives. To assess the effect of ultraviolet-B (UVB) from sun exposure on vitamin D3 [25(OH)D] status and parathyroid hormone (PTH) concentration in elderly Indonesian women.

Methods. A total of 74 elderly women were randomised into the intervention group (receiving UVB from sun exposure at 0.6 minimal erythemal dose/hour on the face and both arms 3 times a week for 6 weeks plus calcium carbonate 1000 mg per day) and a control group (receiving only calcium carbonate 1000 mg per day). Main outcome measures were 25(OH)D, PTH, and serum calcium levels at weeks 0 and 6.

Results. The prevalence of 25(OH)D deficiency was 35%. In the intervention group, the mean 25(OH)D concentration increased from 59 nmol/L to 84 nmol/L, but increased only slightly in the control group. There was no change of PTH levels in either group. In the intervention subgroup with deficient serum levels of 25(OH)D, post-intervention levels increased markedly, but PTH levels decreased only slightly.

Conclusion. Ultraviolet B from sun exposure for 25 minutes, 3 times a week for 6 weeks improves the vitamin D status, but not the PTH levels.

Key words: Aged; Parathyroid hormone; Sunlight; Ultraviolet rays; Vitamin D

[1] Division of Geriatric, Department of Internal Medicine, School of Medicine, University of Indonesia, Indonesia
[2] Division of Endocrine Metabolic, Department of Internal Medicine, School of Medicine, University of Indonesia, Indonesia
[3] Faculty of Public Health, University of Indonesia, Indonesia
[4] Division of Geriatric, Department of Internal Medicine, School of Medicine, University of Indonesia, Indonesia

Correspondence to: Dr S Setiati, Division of Geriatric, Department of Internal Medicine, Faculty of Medicine, University of Indonesia, Jl. Diponegoro no.71, Jakarta 10430, Indonesia. E-mail: s_setiati@plasa.com, s_setiati@yahoo.com

INTRODUCTION

Vitamin D is important for proper development and maintenance of serum calcium levels, skeletal integrity, vascular health, and cancer prevention.[1-3] The hormonal form of vitamin D [1,25 $(OH)_2D_3$] acts through a nuclear receptor with many functions, including calcium and phosphate absorption in the intestine, calcium mobilisation in bone, and calcium reabsorption in the kidney, all of which maintain calcium homeostasis and bone mineralisation in the body.[4-7]

Vitamin D deficiency leads to secondary hyperparathyroidism, increased bone turnover, bone loss, and is also implicated as a cause of hip fracture.[8-12] Furthermore, in the elderly population, vitamin D deficiency is related to lower muscle strength, increased body sway and falls.[13-17] Several factors contribute to vitamin D deficiency in elderly women, including: decreased vitamin D synthesis in the skin, reduced metabolic activity in the liver and kidney, diminished sun exposure, and lower vitamin D intake.[18-22]

Vitamin D deficiency in the elderly population can be treated by supplementing vitamin D (orally or by injection) or increasing vitamin D synthesis with ultraviolet (UV) irradiation using artificial ultraviolet-B (UVB) or sun exposure. Most studies to date have entailed vitamin D supplementation and artificial UV irradiation.[23-26] Only a few studies have reported the effect of sun exposure as a means of increasing vitamin D3 [25(OH)D].[27,28] Whether UVB from

sun exposure in equatorial countries can increase 25(OH)D and decrease parathyroid hormone (PTH) concentration among elderly women was the question we posed. Our study population in Indonesia had the following features: type-4 brown skin, old age, low body mass index, and physiological changes in many organ systems related to vitamin D synthesis. It was reported that 7-dehydrocholesterol in the skin was reduced due to the ageing process.[19,29] Another question to resolve was the proper extent of sun exposure time necessary. Thus, the aim of our study was to obtain the dosage of additional sun exposure (in terms of intensity and duration) necessary to yield a measurable difference in 25(OH)D and PTH levels in elderly Indonesian women.

METHODS

Ours was a randomised clinical trial with a parallel design, entailing additional UVB from deliberate sun exposure as the intervention. The study was undertaken during the wet (rainy) season (February to March 2006) on elderly women from four randomly selected institutionalised care units (two were government run and two were privately run) in two cities, Jakarta and Bekasi.

All the subjects were elderly women (≥60 years) who fulfilled the entry criteria (not bed ridden, willing to enter study, no liver abnormalities, kidney failure or skin cancers, and not in receipt of phenobarbitone or phenytoin). Data pertaining to each subject (demographic characteristics, time and length of sun exposure, 25(OH)D, PTH and serum calcium levels, nutrient intake [calorie, protein, fat, calcium, and vitamin D], height, weight, and skin type) were collected. A UV meter (UV meter model 7.0, Solartech, US) was used to measure UVB intensity from sun exposure, a microtoise and a weighing scale to measure height and weight. A mexameter was used to obtain the melanin index (for skin type), and food recall and food record questionnaires to collect nutritional information. Levels of 25(OH)D and PTH were measured by enzyme-linked immunosorbent assay[30,31] performed in an internationally standardised (ISO 9001) clinical laboratory. As the latter two molecules are very unstable at room temperature, the blood samples were kept in the freezer at -20°C to prevent underestimation before measurement. Vitamin D deficiency was defined as a 25(OH)D level of <50 nmol/L. Increased PTH level was defined as a level of >69 pg/mL. Before the study, all four research assistants received training regarding all necessary procedures as well as the study protocol. Sample size was determined based on the confidence interval of 95%, a study power of 90%, and assuming an effect size of 20%. A total of 80 elderly subjects were recruited.

The study entailed two stages. In the first stage, the intensity of UVB from sun exposure was measured in the randomly selected institutionalised care units in Jakarta and Bekasi, where the study was being carried out. Measurement of sun exposure intensity was performed daily, every hour, from 7 am to 4 pm, over 7 consecutive days . The minimal erythemal dose (MED)/hour as recorded by the UV meter was noted and a mean value was calculated. The time and duration of each exposure were based on the exposure intensity calculated as above. In the second stage, 20 subjects from each institutionalised care unit were randomised to either an intervention or control group. Those in the intervention group were trained to expose their face and both arms to sun for specified durations at specified times, as defined in the first stage. The intervention was carried out 3 times a week for 6 weeks. Subjects were advised to carry out recreational activities and wear sunglasses during each exposure, without application of sunscreen to their face and arms. Subjects in the control group were trained to carry out corresponding activities indoors.

Subjects in both groups were given calcium carbonate 2 x 500 mg per day during 6 weeks of observation. They were questioned in terms of outdoor activities, as well as about the frequency and duration of such activities before entering the study. Data on each subject's nutrient intake were collected before and during the study, based on 24-hour food recall and food weighing (performed by dietitians). Serum 25(OH)D, PTH, and calcium levels were measured before and 6 weeks after sun exposure.

Data were analysed using Stata (version 8.0). Mean values for MED/hour in the first stage was calculated to determine the exact dose for sun exposure. *t*-Test for paired samples were used to compare the difference of 25(OH)D and PTH levels before and after the intervention. *t*-Tests for independent samples were used to compare the difference of 25(OH)D and PTH levels after 6 weeks in the exposed (intervention) and unexposed groups.

Table 1
Mean minimal erythemal dose (MED) per hour at the selected institutionalised care units

Time	Mean MED/hour			
	West Jakarta	North Jakarta	South Jakarta	Bekasi
07.00	0.1	0.1	0.2	0.4
08.00	0.2	0.4	0.4	0.5
09.00	0.6	0.7	0.7	0.6
10.00	0.8	1.2	1.1	0.8
11.00	1.3	1.4	1.8	1.2
12.00	1.1	1.7	2.3	1.6
13.00	0.8	1.3	2.1	1.8
14.00	0.6	0.9	1.3	1.2
15.00	0.2	0.6	0.6	0.4
16.00	0.1	0.5	0.3	0.4

Any p values of <0.05 denoted statistical significance using two-tailed tests.

RESULTS

The lowest intensity of sun exposure was obtained at 7 am. The intensity increased rapidly until 11 am, after which it stabilised somewhat but reached maximum at 2 pm (**Table 1**). According to Holick,[4] the simplest way to obtain vitamin D from sun exposure was by exposing face, arms, and hands for a period equal to 25% of the time that it would take to cause 1 MED. In this study, the highest intensity (more or less 2 MED/hour) occurred at 11 am to 1 pm. Thus it took only ¼ x 60 minutes/2 = 7.5 minutes to increase 25(OH)D concentration. It transpired that high UVB intensity (heat of sunlight) at 11 am to 1 pm caused patients inconvenience and decreased their compliance. The preferred time for sun exposure was at 9 am, when the UVB intensity was 0.6 MED/hour, meaning that it took 25 minutes (¼ x 1 MED/0.6 MED x 60 minutes) for each exposure. All subjects in the intervention group then received 25 minutes for each exposure, 3 times a week for 6 weeks.

The 80 subjects recruited consisted of 42 in the intervention group and 38 controls. Six from the former group were excluded from the analysis due to incomplete sun exposure. A total of 74 subjects completed the study. Most (77%) of the subjects were aged 60 to 75 years and the rest were 76 to 90 years old. The characteristics of the subjects in the two groups are shown in **Table 2**. There were no statistically significant differences in this regard (p>0.05) or the frequency of being outdoors and the duration of sun exposure (p>0.05). Before intervention, 26 (35%) of the subjects had 25(OH)D deficiency and/or an elevated PTH concentration.

After 6 weeks of sun exposure, the mean 25(OH)D concentration increased significantly (p<0.001). Mean values and percentage differences of 25(OH)D concentration before and after this period were significantly higher in the intervention group than in the controls (p=0.01). The mean 25(OH)D and PTH concentrations before and after 6 weeks of sun exposure are shown in **Table 3**. Before commencement, the mean 25(OH)D concentration in the intervention group was lower in older than younger subjects; the mean percentage change was also greater in those who were older.

For further analysis, subjects were divided into those with normal and deficient 25(OH)D concentrations. **Table 4** shows that in the intervention group, 25(OH)D concentration increased significantly, and more so in the deficient than normal group (77% vs 32%). In the deficient group, the PTH level decreased 1% after sun exposure but in the normal group it increased 11%. There was no statistically significant difference between those two groups (p=0.34). Serum calcium levels increased significantly both in the deficient and normal groups (both p<0.001). In the control group, subjects with normal 25(OH)D concentrations had an increase in their mean 25(OH)D concentration after 6 weeks. In the deficient group, mean 25(OH)D concentration increased but not to a statistically significant extent, and mean

Table 2
Demographic and lifestyle characteristics of subjects in the intervention and control groups before treatment period*

Characteristic	Intervention group (n=36)	Control group (n=38)	p Value
Age (years)	71±6	72±8	0.69†
Body mass index (kg/m^2)	2±4	22±4	0.67†
Calcium ion (mmol/L)	1.09±0.03	1.10±0.04	0.12†
Vitamin D3: 25(OH)D (nmol/L)	59.1±20.4	64.8±21.5	0.25†
Parathyroid hormone (pg/mL)	62.9±2.6	58.5±2.1	0.43†
Calorie intake (Kcal)	1469±403	1500±399	0.74†
Protein intake (g)	34±9	35±9	0.69†
Calcium intake (mg)	254±8	242±10	0.59†
Vitamin D intake (μg)	20±8	20±15	0.79†
Outdoor frequency			
Never	1 (3%)	5 (13%)	0.13‡
1/week	14 (39%)	19 (50%)	
2-3/week	20 (56%)	12 (32%)	
>3/week	1 (3%)	2 (5%)	
Length of sun exposure per day (minutes)			
None	1 (3%)	5 (13%)	0.19‡
<30	11 (31%)	14 (37%)	
30-60	10 (28%)	11 (29%)	
>60	14 (39%)	8 (21%)	

* Data are shown in mean±SD or No. of patients (%)
† Independent *t*-test
‡ Chi squared test

Table 3
Mean vitamin D3 [25(OH)D], parathyroid hormone (PTH), and serum calcium concentrations before and after 6-week treatment period

Variable	Week 0	Week 6	Mean change (%)	p Value (dependent *t*-test)
	Mean (SD)			
25(OH)D (nmol/L)				
Intervention group (n=36)	59.1 (20.4)	84.3 (21.2)	+52	<0.001
Control group (n=38)	64.8 (21.5)	71.2 (23.2)	+12	<0.001
p Value (independent *t*-test)		0.01	<0.001	
PTH (pg/mL)				
Intervention group (n=36)	62.9 (2.6)	64.5 (2.5)	+7	0.62
Control group (n=38)	58.5 (2.1)	62.9 (2.7)	+10	0.07
p Value (independent *t*-test)		0.82	0.65	
Serum calcium (mmol/L)				
Intervention group (n=36)	1.09 (0.03)	1.19 (0.04)	+9	<0.001
Control group (n=38)	1.09 (0.04)	1.28 (0.09)	+17	<0.001
p Value (independent *t*-test)			<0.001	

PTH levels increased from 70 to 79 pg/mL.

DISCUSSION

Studies about the effect of UVB irradiation on 25(OH)D concentration were generally carried out in white types-2 and -3 skin colour populations using artificial UVB.[24-26] This is the first study to explore the effect of UVB from sun exposure on 25(OH)D and PTH levels in elderly women with type-4 skin.

Table 4
Vitamin D3 [25(OH)D], parathyroid hormone (PTH), and calcium ion concentrations after treatment period and percentage change from pre-treatment values

Variable	Week 0	Week 6	Mean change (%)	p Value (dependent *t*-test)
	Mean (SD)			
Intervention group				
25(OH)D (nmol/L)				
25(OH)D normal (n=20)	74.9 (12.8)	96.6 (19.2)	+32	<0.001
25(OH)D deficient (n=16)	39.4 (4.6)	68.8 (11.2)	+77	<0.001
p Value (independent *t*-test)		<0.001	<0.001	
PTH (pg/mL)				
25(OH)D normal (n=20)	53.3 (1.7)	57.3(1.7)	+11	0.22
25(OH)D deficient (n=16)	76.1 (2.7)	74.1 (2.9)	-1	0.74
p Value (independent *t*-test)		0.34	0.34	
Calcium ion (mmol/L)				
25(OH)D normal (n=20)	1.09 (0.02)	1.18 (0.04)	+8	<0.001
25(OH)D deficient (n=16)	1.08 (0.03)	1.18 (0.04)	+9	<0.001
p Value (independent *t*-test)		0.88	0.42	
Control group				
25(OH)D (nmol/L)				
25(OH)D normal (n=20)	74.6 (15.7)	81.7 (16.2)	+10	<0.001
25(OH)D deficient (n=16)	37.5 (6.1)	41.9 (11.7)	+12	0.34
p Value (independent *t*-test)		<0.001	0.54	
PTH (pg/mL)				
25(OH)D normal (n=20)	57.2 (21.5)	60.6 (23.3)	+6	0.19
25(OH)D deficient (n=16)	70.0 (24.2)	79.1 (35.7)	+13	0.14
p Value (independent *t*-test)		0.07	0.69	
Calcium ion (mmol/L)				
25(OH)D normal (n=20)	1.10 (0.04)	1.29 (0.09)	+18	<0.001
25(OH)D deficient (n=16)	1.09 (0.03)	1.26 (0.07)	+16	<0.001
p Value (independent *t*-test)		0.29	0.54	

Since there were no data about the intensity of UVB from sun exposure in tropical countries, especially in Indonesia, the first stage of this study aimed to obtain the MED/hour value in our randomly selected institutionalised care units, so as to determine the time and duration of exposure the subjects might need. This study encountered the highest intensity of UVB from sun exposure about 1 hour before and after midday. The exposure duration needed to increase 25(OH)D concentration was longer when subjects were exposed before 11 am and after 1 pm.

The lifestyle of most of the subjects in this study was healthy with regard to vitamin D status. They were not bed-ridden, undertook outdoor activities, and rarely used sunscreen, thus allowing enough sun exposure. Given that sun exposure is the most important source, vitamin D status is expected to relate to geographic location, being better in residents close to the equator compared to those at other latitudes. Vitamin D deficiency is thought to be rare in the Indonesian population as the country is exposed to sunlight all year long. However, our study showed that the incidence of vitamin D deficiency in elderly women was high (35%). In other studies of elderly housebound people and nursing home residents, one quarter and one half respectively were vitamin D deficient.[10,32] Environmental factors such as the frequency of outdoor activity, low levels of sun exposure, and low dietary intake are likely contributing factors to the high prevalence of vitamin D deficiency. The definition of vitamin D deficiency may also affect estimation of its prevalence. However, our study was not designed to explore the risk factors

of vitamin D deficiency. Instead, our aim was to measure the difference of 25(OH)D and PTH levels between exposed and non-exposed groups, before and after sun enxposure.

Data have consistently shown that in the healthy elderly people, 25(OH)D concentration is a function of sun exposure. Cross-sectional and longitudinal studies in white populations living in North Europe and North America exhibit a marked seasonal variation in serum 25(OH)D concentration, being lowest during winter months, and highest in late summer, consistent with the role of sun exposure on 25 (OH)D concentrations.[8,12,33] However, there are certain other factors which also influence sun exposure.[34-36]

Two demographic factors known to contribute to sun exposure are age and skin pigmentation. Ageing significantly affected the capacity of human skin to produce vitamin D.[19] Ageing decreases the amount of 7-dehydrocholesterol produced in the skin, by as much as 75% by the age of 70 years. Moreover, the increase in 25(OH)D concentrations is also lower in people who have greater amounts of melanin. The exposure of Caucasian subjects (type-2 and -3 skins) with 1 MED artificial UV irradiation greatly increased 25(OH)D concentrations by up to 60 fold within 24 to 48 hours of exposure, whereas this dosage did not significantly change 25(OH)D concentrations in Black (type 5-6 skin) subjects.[22] From the perspective of vitamin D synthesis, our study showed that skin photosynthesis and hydroxylation in the liver performed well in Indonesian elderly women with type-4 skin colour, which was expressed by significant increases of 25(OH)D concentration after 6 weeks of sun exposure.

In the deficient group, PTH concentration decreased slightly (1.4%) after 6 weeks of sun exposure. This might have been due to better calcium absorption after sun exposure (serum calcium levels increased from 1.08 to 1.18 mmol/L) or the result of calcium supplementation. In the deficient unexposed group, PTH concentration increased after 6 weeks (70 to 79 pg/mL), although serum calcium levels increased from 1.09 to 1.26 mmol/L. From which it may be assumed that the increase of serum calcium levels was due to bone resorption through heightened secondary hyperparathyroidism. Presumably, the low 25(OH)D concentration in this group was insufficient for the formation of enough $1.25(OH)_2D_3$ needed to increase intestinal calcium absorption.

Other studies which used artificial UVB and vitamin D supplementation found greater decreases in PTH concentration.[14,23] However, there are some important differences between the study by Chel et al[23] and ours. Thus, all their subjects had severe vitamin D deficiency (25(OH)D of <20 nmol/L) and less skin pigmentation (type-2 and -3 skin). They also used artificial UVB exposure from a reliably stable and intense source.[23] Both studies encountered the same phenomenon, namely the lower the pre-exposure 25(OH)D concentration, the greater the increase of 25(OH)D concentration post-exposure.

Pfeifer et al[14] compared elderly women given 1200 mg/day calcium and 800 IU/day vitamin D supplementation for 8 weeks with those who were given 1200 mg/day calcium supplementation alone. They found that 25(OH)D increased 72% and PTH decreased 18% in calcium and vitamin D supplementation group. As in our study, they found that the 25(OH)D concentration increased significantly more in the deficient group (77% vs 72%).[14] However, the PTH concentration decreased less in our study than that in theirs (1.4% vs 18%). Besides, the shorter duration of intervention (6 weeks of sun exposure in our study vs 8 weeks of supplementation in theirs), as well as other differences in the two populations (kidney and parathyroid gland function, race and ethnicity) may explain the contrasting PTH level responses.

The increase of 25(OH)D concentration after sun exposure indicated that our subjects achieved good vitamin D synthesis in skin, although they were elderly with type-4 skins. Further vitamin D synthesis in the liver was also good. Regrettably, the increase of 25(OH)D concentration was not followed by decreases in PTH concentration.

A limitation of our study was that the UV meter measures UVB as well as UVA. In the early morning more UVA gets through the stratosphere than UVB, and thus the total UVB we measured might have been a mix of both types.

In conclusion, vitamin D deficiency was found in 35% of Indonesian elderly women in institutionalised care. Receipt of UVB from sun exposure for 25

minutes, 3 times a week, for 6 weeks improved the vitamin D status, but not the PTH levels. It may take more than 6 weeks of sun exposure to decrease PTH levels. The optimal exposure time is 1 hour before and after midday. Further studies with longer periods of observation are needed to compare the effect of sun exposure and vitamin D supplementation on 25(OH)D concentration, PTH levels, muscle strength, and bone mass density. Further investigations are also needed to explore into the timing and duration of sun exposure in relation to 25(OH)D concentration, in the context of different geographical locations, climatic conditions, and seasons.

References

1. Peterlik M, Cross HS. Vitamin D and calcium deficits predispose for multiple chronic diseases. *Eur J Clin Invest* 2005;35:290-304.
2. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004;80(6 Suppl):S1678-88.
3. Jones G, Strugnell SA, DeLuca HF. Current understanding of the molecular actions of vitamin D. *Physiol Rev* 1998;78:1193-231.
4. Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr* 2004;79:362-71.
5. Garfia B, Canadillas S, Canalejo A, Luque F, Siendones E, Quesada M, et al. Regulation of parathyroid vitamin D receptor expression by extracellular calcium. *J Am Soc Nephrol* 2002;13:2945-52.
6. Brown AJ, Dusso A, Slatopolsky E. Vitamin D. *Am J Physiol* 1999;277:F157-75.
7. Holick MF. Vitamin D: underappreciated D-light hormone that is important for skeletal and cellular health. *Curr Opin Endocrinol Diabetes* 2002;9:87-98.
8. Chapuy MC, Schott AM, Garnero P, Hans D, Delmas PD, Meunier PJ. Healthy elderly French women living at home have secondary hyperparathyroidism and high bone turnover in winter. EPIDOS Study Group. *J Clin Endocrinol Metab* 1996;81:1129-33.
9. Serhan E, Holland MR. Relationship of hypovitaminosis D and secondary hyperparathyroidism with bone mineral density among UK resident Indo-Asians. *Ann Rheum Dis* 2002;61:456-8.
10. Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, et al. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. *J Clin Endocrinol Metab* 2001;86:1212-21.
11. Pfeifer M, Minne HW. Vitamin D and hip fracture. *Trends Endocrinol Metab* 1999;10:417-20.
12. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. *Endocr Rev* 2001;22:477-501.
13. Visser M, Deeg DJ, Lips P; Longitudinal Aging Study Amsterdam. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *J Clin Endocrinol Metab* 2003;88:5766-72.
14. Pfeifer M, Begerow B, Minne HW, Abrams C, Nachtigall D, Hansen C. Effects of a short-term vitamin D and calcium supplementation on body sway and secondary hyperparathyroidism in elderly women. *J Bone Miner Res* 2000;15:1113-8.
15. Bischoff HA, Stahelin HB, Dick W, Akos R, Knecht M, Salis C, et al. Effects of vitamin D and calcium supplementation on falls: a randomized controlled trial. *J Bone Miner Res* 2003;18:343-51.
16. Bischoff-Ferrari HA, Dawson-Hughes B, Willet WC, Staehelin HB, Bazemore MG, Zee RY, et al. Effects of vitamin D on falls: a meta-analysis. *JAMA* 2004;291:1999-2006.
17. Verhaar HJ, Samson MM, Jansen PA, de Vreede PL, Manten JW, Duursma SA. Muscle strength, functional mobility and vitamin D in older women. *Aging (Milano)* 2000;12:455-60.
18. Parikh SJ, Edelman M, Uwaifo GI, Freedman RJ, Semega-Janneh M, Reynolds J, et al. The relationship between obesity and serum 1,25-dihydroxy vitamin D concentrations in healthy adults. *J Clin Endocrinol Metab* 2004;89:1196-9.
19. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. *J Clin Invest* 1985;76:1536-8.
20. Need AG, Morris HA, Horowitz M, Nordin C. Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. *Am J Clin Nutr* 1993;58:882-5.
21. Nakamura K, Nashimoto M, Okuda Y, Ota T, Yamamoto M. Fish as a major source of vitamin D in the Japanese diet. *Nutrition* 2002;18:415-6.
22. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigment reduces the capacity of skin to synthesise vitamin D3. *Lancet* 1982;1:74-6.
23. Chel VG, Ooms ME, Popp-Snijders C, Pavel S, Schothorst AA, Meulemans CC, et al. Ultraviolet irradiation corrects vitamin D deficiency and suppresses secondary hyperparathyroidism in the elderly. *J Bone Miner Res* 1998;13:1238-42.
24. Adams JS, Clemens TL, Parrish JA, Holick MF. Vitamin-D synthesis and metabolism after ultraviolet irradiation of normal and vitamin-D-deficient subjects. *N Engl J Med* 1982;306:722-5.
25. Chuck A, Todd J, Diffey B. Subliminal ultraviolet-B irradiation for the prevention of vitamin D deficiency in the elderly: a feasibility study. *Photodermatol Photoimmunol Photomed* 2001;17:168-71.
26. Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. *N Engl J Med* 1997;337:670-6.
27. Reid IR, Gallagher DJ, Bosworth J. Prophylaxis against vitamin D deficiency in the elderly by regular sunlight exposure. *Age Ageing* 1986;15:35-40.
28. Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, et al. Vitamin D3 and calcium to prevent hip fractures in the elderly women. *N Engl J Med* 1992;327:1637-42.
29. Gilchrest B. Aging of the skin. In: Hazzard WR, Blass JP, Halter JB, Ouslander JG, Tinetti M. *Principles of geriatric medicine and gerontology*. US: McGraw-Hill: 186-9.
30. IDS Inc. *Immuno Diagnostic System*, US. 2004:3-9.
31. *Immulite/Imulite 1000 intact PTH*. PILKPP-13.2005-05-02.
32. Gloth FM 3rd, Gundberg CM, Hollis BW, Haddad JG Jr, Tobin JD. Vitamin D deficiency in homebound elderly persons. *JAMA* 1995;274:1683-6.
33. Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr* 1998;67:1232-6.
34. Agarwal KS, Mughal MZ, Upadhyay P, Berry JL, Mawer EB, Puliyel JM. The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. *Arch Dis Child* 2002;87:111-3.
35. Glerup H. Vitamin D deficiency among immigrants [in Danish]. *Ugeskr Laeger* 2000;162:6196-9.
36. Matsuoka LY, Ide L, Wortsman J, MacLaughlin JA, Holick MF. Sunscreens suppress cutaneous vitamin D3 synthesis. *J Clin Endocrinol Metab* 1987;64:1165-8.