Vitamin D levels for optimum bone health

Masud F

ABSTRACT

Introduction: Vitamin D deficiency was assessed previously on the basis of rickets and osteomalacia, which represent an extreme end of the spectrum. As a result of this, many clinically-asymptomatic patients go undetected. As vitamin D deficiency results in secondary hyperparathyroidism, we propose to use the normalisation of intact parathyroid hormone (iPTH) as a surrogate marker for assessing the adequacy of vitamin D nutrition.

Methods: A descriptive study was undertaken on 195 premenopausal Pakistani women. 25-hydroxy-cholecalciferol and iPTH levels were measured by standard laboratory techniques.

Results: The minimum level of vitamin D required to keep iPTH below 53 pg/dL was found to be 16 ng/ml with a 95 percent confidence interval of 13.8 and 18.2. Existing normal range is 9-36 ng/ml.

Conclusion: Normalisation of iPTH if taken as a criterion for judging vitamin D deficiency can lead to detection of clinically-asymptomatic patients. The simplicity, low cost of correction, and the potential beneficial skeletal and non-skeletal consequences of doing so makes it essential that this criterion be used to redefine the optimal vitamin D levels. This should be internationally standardised and made available to clinicians.

Keywords: 25-hydroxy-cholecalciferol, osteomalacia, rickets, secondary hyperparathyroidism, vitamin D levels

Singapore Med J 2007; 48(3):207–212

Department of Medicine, Services Institute of Medical Sciences, Services Hospital, Jail Road, Lahore 54700, Pakistan

Masud F, MBBS, FRCP
Professor

Correspondence to:
Dr Faisal Masud
Tel: (92) 42 920 3425
Fax: (92) 42 920 3426
Email: dr.faisalmasud@gmail.com

INTRODUCTION

Bone health and calcium homeostasis are mainly dependent upon adequate vitamin D levels. Severe vitamin D depletion manifests as osteomalacia in adults. However, mild to moderate deficiency is common among asymptomatic individuals, or may produce only vague symptoms of bone or muscle pain and tenderness. In modern practice, most cases are detected biochemically in high-risk individuals before clear symptoms are apparent. Vitamin D deficiency is usually diagnosed by a lower than normal 25-hydroxy-cholecalciferol (25-OH-D_3) levels.

The normal value of vitamin D varies considerably between populations, and is dependent upon many geographical, racial and cultural factors. However, in the absence of primary parathyroid pathology, low levels of vitamin D stimulates the production of parathyroid hormone (PTH). Thus, a high PTH value can be used as a surrogate marker of vitamin D deficiency. Women of Asian origin are among the most commonly affected population, even in the Western literature. However, no reliable local data is available for the vitamin D status in Pakistani women. The objective of the present study is to assess the level of vitamin D in pre-menopausal women of Pakistani origin complaining of generalised aches and pains, and to attempt to define the minimum level of vitamin D required for prevention of secondary hyperparathyroidism.

METHODS

This was a descriptive study which included 195 pre-menopausal women of Pakistani origin between the ages of 13 and 52 years. The study was carried out in Lahore. Lahore lies at latitude 31°33'N and longitude 71°26'E. In this region, average sunshine is 255 hours/month during summer (April–September) and 217 hours/month during winter (October–March). The study was conducted from August 2003 to July 2005 at the Services Institute of Medical Sciences. Participants were recruited through convenient sampling on an outdoor basis, after completion of a pre-evaluation per forma.

Inclusion criteria were:

1. Pre-menopausal females between the ages of 13 and 52 years, with regular menstrual cycles and normal routine clinical examination.
2. Symptoms of generalised aches and pains.
3. Born in Pakistan with local ancestry, and born and brought up in an urban setting.

Exclusion criteria included:

1. Intake of calcium/vitamin D supplements during the

past four weeks.

2. Liver enzymes more than three times the upper normal limit; renal impairment with blood urea more than twice the upper normal limit

3. Clinical evidence of hyperparathyroidism or serum calcium more than 10 mg/dL.

4. Subjects with phosphate levels greater than 5 mg/dL, alkaline phosphatase more than twice the upper limit.

5. Subjects on anticonvulsant therapy.

6. History of diarrhoea of more than six months duration.

7. Failure to sign the informed consent.

Overnight (eight hours) fasting samples were taken to determine 25-OH-D_3, intact PTH (iPTH), serum calcium, serum phosphate, alkaline phosphatase, and urea and liver enzymes. 25-OH-D_3 was measured by immunosorbent radioimmunoassay technique on 3.5 ml of clotted blood (reference kit no: 68100E Diasorin Stillwater, MN, USA). Assay precision was evaluated at Diasorin by testing four control levels. The total imprecision ranged from 9.4% to 11.1%.

iPTH was measured on 3.5 ml of ethylene-diaminetetraacetic acid-added blood by the immulite technique using DPC (Diagnostic Product Corporation, Los Angeles, CA, USA) chemiluminescent enzyme-labelled immunometric assay. Interassay precision (pg/ml) was found to have a range of 8.6%–9.0%. The reference range for vitamin D assay was taken as 9–36 ng/ml and the normal range for iPTH as 9–53 pg/dL.

RESULTS

This descriptive study was performed at Services Hospital, Lahore from August 2003 to June 2005 during both summer and winter months. A total of 201 pre-menopausal female patients were enrolled. Six were excluded from the study because of reasons given in Table I. The mean vitamin D and iPTH levels of the study population was calculated (Table II, Fig. 1). The mean vitamin D level was 15.20 ng/ml (standard deviation [SD] = 33), while mean iPTH level came out to be 73.89 pg/dL (SD = 47.46). Vitamin D showed an inverse relationship with iPTH levels in the whole sample, which just failed to reach the level of statistical significance.

Out of 195 patients enrolled, 101 came during the summer months. The remaining 94 patients were recruited during the winter months (Table III). The mean vitamin D level in the "summer group" was 15.70 ng/ml, with a mean iPTH value of 73.059 pg/dL. Among winter recruits, the mean vitamin D level was 14.75 ng/ml, and mean iPTH level was calculated to be 73.695 pg/dL. The mean vitamin D levels were estimated for different months (Table IV, Fig. 2). The highest mean value for vitamin D was found during the months of September and October, while minimum levels were seen during January and February.

The study population was subdivided into age groups and analysed (Table V). The maximum numbers of subjects were between 33 and 42 years of age. The mean vitamin D levels fell between 13.68–16.24 ng/ml, whereas the mean value of iPTH was more than 55 pg/dL in all the age groups studied. Taking the traditional vitamin D adequacy value of 9 ng/ml, the group showing vitamin D

Table I. Details of excluded subjects.

Cause of exclusion		Number
Elevated ALT	> 3* normal	3
Elevated urea	> 3* normal	2
Elevated phosphate	> 5 mg/dL	1

Table II. Mean vitamin D and iPTH values.

Number of subjects	Mean vitamin D (ng/ml)	Mean iPTH (pg/dL)	Pearson correlation coefficient (2-tailed)
195	15.2022 ± 13.3775	73.8921 ± 47.4699	0.055

Vitamin D levels show an inverse relationship with iPTH levels in the whole sample, which just failed to reach the level of statistical significance.

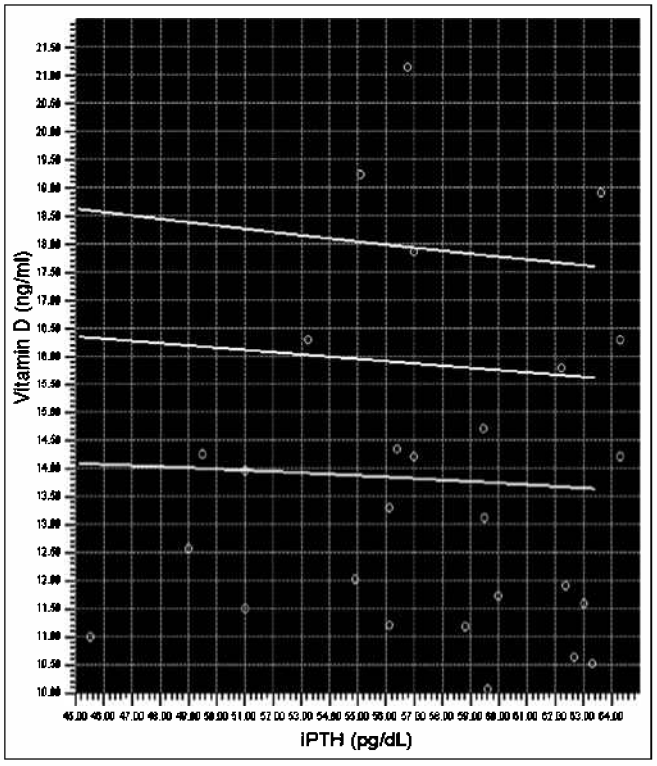

Fig. 1 Graph shows relationship between vitamin D and iPTH levels.

Mean vitamin D level was 15.20 ng/ml (SD = 13.33), while mean iPTH level was 73.89 pg/dL (SD = 47.46). Vitamin D shows an inverse relationship with iPTH levels in the whole sample, which just failed to reach the level of statistical significance.

deficiency (levels less than 9 ng/ml) was analysed on the basis of seasonal variation (Table VI). Significantly lower levels of vitamin D (and correspondingly high iPTH), were observed during the summer months.

The vitamin D deficient group was divided into age groups. Subjects with iPTH above the normal range (n = 145) were also separated and analysed (Table VII). The minimum mean levels of vitamin D were seen in subjects between 23 and 32 years of age. Out of 195, a total of 60 subjects had vitamin D levels below 9 ng/ml. In contrast, iPTH was more than the upper normal limit (53 pg/dL) in 145 study cases, thus a one to one relationship was not observed between the two variables.

In an attempt to define hypovitaminosis D on the basis of secondary increase in PTH levels (more than 53 pg/dL), the values for vitamin D and iPTH were plotted on a scatter plot (Fig. 3). The minimum level of vitamin D required to keep iPTH below 53 pg/dL was found to be 16 ng/ml with a 95% confidence interval of 13.8 and 18.2.

DISCUSSION

Vitamin D is an essential nutrient for optimal bone health. It is obtained partly from diet, but a significant contribution to the total pool is made by the skin, where de-novo synthesis of vitamin D from its precursor 17-dehydrocholestrol occurs on exposure to ultraviolet-B radiation.[1] Strictly speaking, therefore, it is not a vitamin but a hormone which is converted by two successive hydroxylations in the liver and kidney to its active metabolite 1,25-dihydroxy-cholecalciferol (1,25 $(OH)_2$-D_3). This form of vitamin D enhances the intestinal absorption of calcium, which is needed for bone mineralisation.

While severe depletion of vitamin D manifests as osteomalacia in adults, and rickets in children, mild to moderate deficiency is common.[2] This may be totally asymptomatic; or the patient may present with vague aches and bone pains. Early detection is important, as deficiency is associated with an enhanced risk of osteoporosis and skeletal fractures.[3] 25-OH-D_3, which is the intermediate metabolite of vitamin D, is routinely used to estimate vitamin D adequacy. This is because its plasma levels are 100 times higher than those of 1,25 $(OH)_2$-D_3, and also because plasma levels of 1,25 $(OH)_2$-D_3 can be normal or even elevated in mild to moderate osteomalacia.[4,5]

The prevalence of hypovitaminosis D has been extensively reported in the West,[6–11] with women of Asian origin being among the most commonly affected groups.[12,13] However, to date, no reliable local data is available for Pakistani women. In this study, we have attempted to define vitamin D status among Pakistani women of child-bearing age. For assessment of vitamin D sufficiency, 25-OH-D_3 levels are commonly employed.

Table III. Group statistics based on seasonal variation.

Season	Summer n = 101	Winter n = 94
Mean vitamin D (ng/ml)	15.706 ± 13.985	14.756 ± 9.906
Mean iPTH (pg/dL)	73.059 ± 55.69	73.695 ± 1.82

Table IV. Vitamin D levels during summer and winter months.

Months	Sunshine hours	n	Mean vitamin D (ng/ml)
January–February	202	36	12.7 ± 7.4#
March–April	254	44	16.7 ± 16.3
May–June	292	25	15.0 ± 10.8
July–August	235	28	14.2 ± 10.0
September–October	247	14	18.0 ± 11.0*
November–December	219	45	17.8 ± 11.0

*Highest mean levels of vitamin D; #Lowest mean levels of vitamin D.

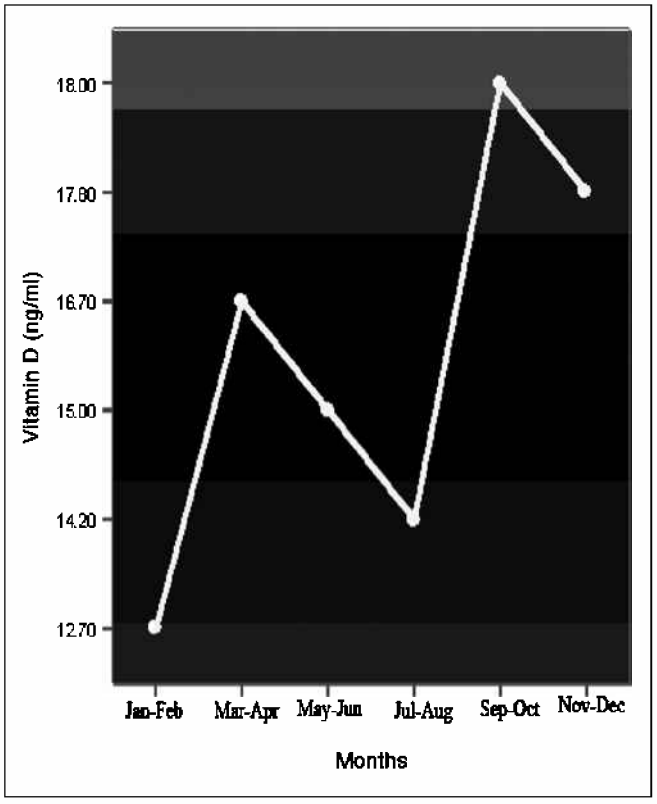

Fig. 2 Graph shows bimonthly variation of vitamin D levels.

Highest mean value for vitamin D was found during the months of September and October, while minimum levels were seen during January and February.

Table V. Distribution of patients according to age, and their mean iPTH and vitamin D levels.

Age range (years)	No. of patients (%)	Mean vitamin D (ng/ml)	Mean iPTH (pg/dL)
13–22	9 (4.37)	16.24 ± 12.54	55.26 ± 26.98
23–32	39 (19.67)	13.68 ± 12.12	82.67 ± 56.58
33–42	82 (40.98)	15.33 ± 13.39	72.48 ± 53.37
43–52	65 (34.97)	15.47 ± 11.12	72.36 ± 29.01

Table VI. Vitamin D deficiency and seasonal variation.

Season	Summer	Winter	Significance level
Mean vitamin D in population with vitamin D level < 9 ng /ml (ng/ml)	4.42 ± 3.26 (n = 28)	6.90 ± 2.49 (n = 32)	0.001
Mean iPTH in population with vitamin D level < 9 ng /ml (pg/dL)	101.9 ± 92.2 (n = 28)	73.5 ± 25.8 (n = 32)	0.001

Table VII. Abnormal vitamin D and iPTH levels and age distribution.

Age distribution (years)	Low vitamin D (ng/ml) n = 60	High iPTH (pg/dL) n = 145
13–22	7.45 ± 0.64 (n = 4)	89.65 ± 10.81 (n =2)
23–32	4.71 ± 0.43 (n =12)	90.69 ± 58.27 (n =32)
33–42	6.18 ± 2.97 (n =30)	86.42 ± 55.83 (n =60)
43–52	5.20 ± 3.40 (n =14)	80.33 ± 26.88 (n =51)

However, 25-OH-D_3 alone is not a good marker of vitamin D adequacy. Firstly, because the value of vitamin D varies considerably between populations (depending upon various factors such as clothing, diet, latitude, race, etc),[14] and we do not have a local reference range. Secondly, long before diseases with low serum vitamin D values manifest clinically, the lowered calcium levels due to mild to moderate vitamin D deficiency cause secondary stimulation of PTH.[4] Overt osteomalacia and reduced blood levels of vitamin D_3 appear only when vitamin D is severely depleted, and secondary hyperparathyroidism fails to compensate for this deficiency state. Thus, in the absence of primary parathyroid pathology, a high level of PTH indicates vitamin D inadequacy even if its blood levels are apparently normal. This has brought up the question of redefining vitamin D deficiency as the lowest threshold value for plasma 25-(OH)-D_3 in ng/ml that prevents secondary hyperparathyroidism. Use of this "physiological" parameter for the definition of vitamin D adequacy, rather than the absence of "pathological" rickets/osteomalacia, has been proposed already.[15,16]

In the present study, 195 women of child-bearing age, presenting with 'aches and pains', were assessed for vitamin D adequacy using both 25-OH-D_3 and iPTH. All subjects were of Pakistani race and origin, living in an urban setup, and with similar religious and cultural practices. All were exposed roughly to the same amount

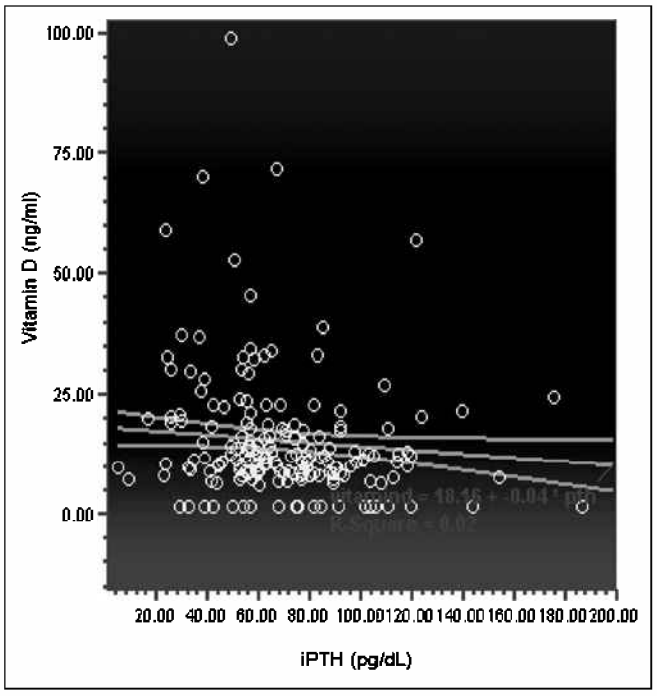

Fig. 3 Scatter plot graph shows relationship between vitamin D and iPTH levels.

The minimum level of vitamin D required to keep iPTH below 53 pg/dL was found to be 16 ng/ml with a 95% confidence interval of 13.8 and 18.21.

of sunshine at latitude and longitude of 31°33'N and 71°26'E, respectively. The exact degree of sun exposure and daily intake of vitamin D and calcium were obtained by self reporting.

The mean vitamin D level of our study population (15.20 ± 13.37 ng/ml) was found to be well within the reference range (9–36 ng/ml); while the mean iPTH level (73.89 ± 47.46 pg/dL) was clearly above the upper normal limit. Moreover, the prevalence of hyperparathyroidism was 43.5% more than that of hypovitaminosis D alone. This lack of 1:1 relationship emphasises the need for redefining vitamin D adequacy on the basis of normal iPTH values. The minimum 25-OH-D_3 level required to keep iPTH within normal limits was found to be 16.0 ± 2.0 ng/ml.

Analysis based on age subgroups showed minimum levels of vitamin D (and correspondingly highest iPTH) in subjects between 23 and 32 years of age. This tallies with the period of maximum fertility in Pakistan, and women of this age group are largely house-bound. Breastfeeding is commonly practised and may also be a factor. Maximum vitamin D levels were observed among females of 13–22 years, the youngest recruited age group, mostly students and unmarried women with relatively greater outdoor hours. To find out the effect of local seasonal variation on vitamin D status, levels were analysed for different months of the year. In contrast to western studies, minimum vitamin D levels (with maximum iPTH values) were observed during peak summer months. This can be attributed to the hot temperatures (going up to and beyond 50°C), which limit sun exposure to the minimum possible, particularly from May to August. Thus effective sunlight remains largely unavailable during the summer. Application of sunscreens may have also played a role.[17]

Even though there is no such period as "vitamin D winter" in this part of the world, the relatively lesser dip seen in November and December was probably due to protective clothing worn during the cooler months. Due to ambient temperatures, spring and autumn are the seasons best suited for outdoor activities in this region. Our data corresponds to these periods of maximum sunlight exposure, showing peaks of vitamin D during March and April, and then September and October.

To conclude, 81% of the women in the study showed evidence of vitamin D deficiency, with mean vitamin D_3 levels of 15.20 ± 13.37 ng/ml. They had all come with vague complaints of aches and pains, without biochemical markers of overt osteomalacia. However, calculation of exact prevalence requires larger scale studies. Seasonal variation should be confirmed through follow-up of individual subjects through different months of the year. Still, this study is important, as it indicates the need for further research regarding vitamin D status in the local population. In addition, this study, like many others done previously but in different age groups and populations, has shown that the existing lower normal limit of vitamin D (9 ng/ml) is not adequate to keep iPTH within the normal range, i.e. below 53 pg/dL. The minimum 25-(OH)-D value found to keep iPTH below 53 pg/dL in our study was 16.0 ± 2.0 ng/ml. Larger studies to confirm the inaccuracy of the existing lower limit on the basis of iPTH could perhaps lead to revision of the current reference range for 25-(OH)-D_3 blood levels. Consequent early detection of vitamin D deficiency would allow for effective prophylaxis, and thus help prevent overt osteomalacia and fractures.

There are several ways to improve vitamin D status in the general population: (1) fortification of food; (2) yearly vitamin D injections; (3) oral vitamin D and calcium supplementation; (4) regular sunlight exposure. Given what may appear to be an epidemic of vitamin D deficiency, the good thing is that the problem is now out in the open, and physicians seem much more aware of it than they had been in the past. The simplicity and low cost of correction, and the potential beneficial skeletal and non-skeletal consequences of doing so, make it essential that the medical community should define a threshold for optimal vitamin D status using accurate, reproducible assays. These assays must subsequently be internationally standardised and made available to practising clinicians.

REFERENCES

1. Higdon J. Vitamin D. In: Micronutrient Information Center [online]. Available at: lpi.oregonstate.edu/infocenter/vitamins/vitaminD/index.html. Accessed November 1, 2006.
2. Parikh N, Eskridge R, Hill J, Phillips E, Rao D. Prevalence of vitamin D depletion among subject seeking advice on osteoporosis: a five-year cross-sectional study with therapeutic and health implications (abstract). J Bone Miner Res 2002; 17 suppl 1:S201.
3. Bringhurst TR, Demay MB, Kronenberg HM. Mineral metabolism. In: Larson PR, Kronenberg HM, Melmand S, Polonsky KS, eds. Williams Textbook of Endocrinology. 10th ed. Philadelphia: WB Saunders, 2003:1317-20.
4. Parfitt AM. Osteomalacia and related disorders. In: Avioli LV, Krane SM, eds. Metabolic Bone Diseases and Clinically Related Disorders. San Diego: Academic Press, 1998:3278-86.
5. 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.
6. Oliveri B, Plantalech L, Bagur A, et al. High prevalence of vitamin D insufficiency in the elderly population in Argentina (abstract). J Bone Miner Res 2002; 17 suppl 1:S354.
7. Sackrison JL, Body JJ, Ersfeld DL, et al. 2002. Hypovitaminosis D in an urban European population (abstract). J Bone Miner Res 2002; 17 suppl 1:S459.
8. Saraiva GL, Cendoroglo MS, Ramos JR. 2002. High prevalence of vitamin D deficiency and hyperparathyroidism in elderly population from City of Sao Paulo, Brazil (abstract). J Bone Miner Res 2002; 17 suppl 1: S458.
9. Hasid R, Tamir A, Raz B, Segal E, Ish-Shalom S. Impaired vitamin D status in young Israeli women (abstract). J Bone Miner Res 2002; 17 suppl 1:S267.
10. Ryan PJ. Hypovitaminosis D in clinical practice (abstract). J Bone Miner Res 2002; 17 suppl 1:S316.
11. Feldkamp J, Schott M, Fritzen R, Scherbaum WA. Osteomalacia in immigrants from the Middle East. Bone 2001; 28 suppl:S204.
12. Serhan E, Holland MR. The effect of calcium and vitamin D supplementation in Indo-Asians suffering from hypovitaminosis D with or without secondary hyperparathyroidism (abstract). Bone 2001; 28 suppl:S205.
13. Iqbal SJ, Davies TD, Muhlbayer S, McHugh G, Badawy O. Biochemical measures and bone mineral density in Asian patients with

severe vitamin D deficiency [abstract]. Bone 2001; 28 suppl:S205.
14. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. Am J Clin Nutr 1995; 61 (3 suppl):638S-645S.
15. Trivedi DP, Doll R, Khaw KT. Effect of four monthly vitamin D_3 (cholecalciferol) supplementation on fractures and mortality in men and women living in community: randomised double blind controlled trial. BMJ 2003; 326:469-74. Comment in: Evid Based Nurs 2003; 6:113. J Fam Pract 2003; 52:431-5.
16. Johnell O, Gullberg B, Kanis JA, et al. Risk factors for hip fracture in European women: the MEDOS study. Mediterranean Osteoporosis Study. J Bone Miner Res 1995; 10:1802-15.
17. 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. Erratum in: Am J Clin Nutr 2004; 79:890.