

Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi^{1,2}

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ABSTRACT

Background: Despite abundant sunlight, rickets and osteomalacia are prevalent in South Asian countries. The cause of this paradox is not clear.

Objective: The objective was to assess 25-hydroxyvitamin D [25(OH)D] status and its functional significance in apparently healthy subjects residing in Delhi, a city in the northern part of India.

Design: Serum 25(OH)D, total calcium, inorganic phosphate, alkaline phosphatase, intact parathyroid hormone, and 1,25-dihydroxyvitamin D [1,25(OH)₂D] were measured in groups of healthy subjects who differed with respect to variables relevant to vitamin D and bone mineral metabolic status, such as direct sunlight exposure, season of measurement, skin pigmentation, dietary calcium and phytate contents, and altered physiologic states such as pregnancy and neonatal age.

Results: All groups except one with maximum direct sunlight exposure had subnormal concentrations of 25(OH)D. The 25(OH)D-deficient groups tended to have an imbalance in bone mineral metabolic homeostasis when exposed to winter weather and low dietary calcium and high dietary phytate, with significantly low calcium and elevated intact parathyroid hormone concentrations, chemical osteomalacia, or both. Increased values of 1,25(OH)₂D during pregnancy did not help correct the imbalance in bone mineral metabolic homeostasis.

Conclusion: Healthy subjects with low 25(OH)D concentrations are at risk of bone mineral metabolic imbalance when exposed to factors that strain bone mineral homeostasis. *Am J Clin Nutr* 2000;72:472–5.

KEY WORDS 25-Hydroxyvitamin D, 25(OH)D, calcium homeostasis, South Asians, pregnancy, newborn, skin pigmentation, vitiligo, sunshine, intact parathyroid hormone, 1,25-dihydroxyvitamin D, 1,25(OH)₂D

INTRODUCTION

In 1973 Hodgkin et al (1) reported that osteomalacia resulting from vitamin D deficiency was uncommon among Punjabis in India, in contrast with Punjabis in Britain. The investigators explained the disparity by the difference in sunlight exposure in the 2 populations. Since then, no studies have assessed the vitamin D status of healthy persons living in the tropical and subtropical regions of India and other South Asian countries with

use of modern techniques and indexes (2–5). Such studies are relevant in view of the prevalence of osteomalacia and rickets in the region (6) and the now known role of melanin in inhibiting the dermal synthesis of vitamin D (4, 7).

Previously, we reported very low values of serum 25-hydroxyvitamin D [25(OH)D] both in healthy subjects and in patients with hyperparathyroidism from the city of Delhi (8). Vitamin D deficiency was also reported among pregnant women in Karachi, Pakistan (9). In the present study, we measured serum 25(OH)D, 1,25-dihydroxyvitamin D [1,25(OH)₂D], intact parathyroid hormone (PTH-i), total calcium, inorganic phosphate, and alkaline phosphatase activity in 6 groups of healthy subjects from Delhi who were selected on the basis of differentiating features relevant to vitamin D status.

SUBJECTS AND METHODS

After we obtained informed consent, we consecutively studied 123 healthy subjects belonging to the following 6 distinct groups: physicians and nurses, who were studied both in the winter and in the summer ($n = 11$ men and 8 women); soldiers ($n = 31$ men); depigmented persons ($n = 10$ men and 5 women); 9 with vitiligo universalis and 6 with albinism; pregnant women belonging to a poor socioeconomic class ($n = 29$; annual income <30,000 rupees/y); and the newborn children of the pregnant women ($n = 29$). Subjects were evaluated clinically to rule out metabolic bone disease, chronic hepatic and renal disorders, and other vitamin and mineral deficiencies. Subjects taking vitamin and mineral supplementation or any drugs or sunscreens were excluded.

Three groups were studied in the winter: the soldier group, the depigmented group, and the physician and nurse group, and 3 groups were studied in the summer: the pregnant group, the newborn group, and the physician and nurse group. The physician and nurse group was studied in both winter and summer to evaluate the effect of seasonal variation on vitamin D status.

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TABLE 1Clinical characteristics, sun exposure, and serum concentrations of vitamin D and related variables in the study groups¹

Variable	Soldiers (winter) (n = 31 M)	Physicians and nurses (winter) (n = 11 M, 8 F)	Depigmented persons (winter) (n = 10 M, 5 F)	Physicians and nurses (summer) (n = 11 M, 8 F)	Pregnant women (summer) (n = 29 F)	Newborns (summer) (n = 16 M, 13 F)
Age (y)	25 ± 5 ^b	23 ± 5 ^b	43 ± 16 ^a	24 ± 4 ^b	23 ± 3 ^b	—
BMI (kg/m ²)						
Men	21.2 ± 2.0 ^b	22.3 ± 2.8 ^b	24.2 ± 4.4 ^a	22.3 ± 2.8 ^b	—	—
Women	—	20.0 ± 1.7 ^b	24.0 ± 5.0 ^a	20.0 ± 1.7 ^b	18.7 ± 2.4 ^b	—
Calcium intake (mg/d)	1104 ± 666 ^a	879 ± 165 ^{a,b}	980 ± 300 ^{a,b}	879 ± 165 ^{a,b}	345 ± 78 ^b	—
Dietary phytate:calcium	0.96 ± 0.40 ^b	0.74 ± 0.42 ^b	0.82 ± 0.5 ^b	0.74 ± 0.42 ^b	1.69 ± 1.1 ^a	—
Sun exposure (min/d)	370 ± 30 ^a	25 ± 5 ^b	5 ± 5 ^c	25 ± 5 ^b	25 ± 5 ^b	—
25(OH)D (nmol/L)	47.17 ± 11.73 ^a	7.98 ± 3.49 ^d	18.2 ± 11.23 ^{b,c}	17.97 ± 7.98 ^{b,c}	21.9 ± 10.73 ^b	16.72 ± 4.99 ^c
1,25(OH) ₂ D (pmol/L)	ND	ND	ND	93.6 ± 29.0 ^b	160.8 ± 59.0 ^a	114.5 ± 55.4 ^b
PTH-i (ng/L)	17.6 ± 4.8 ^b	38.8 ± 18.2 ^a	35.3 ± 12.6 ^a	ND	ND	ND
Total calcium (mmol/L)	2.35 ± 0.17 ^a	2.17 ± 0.10 ^b	2.22 ± 0.10 ^a	2.25 ± 0.3 ^{a,b}	1.92 ± 0.27 ^c	2.17 ± 0.10 ^b
Inorganic phosphate (mmol/L)	1.22 ± 0.13	1.16 ± 0.16	1.19 ± 0.13	1.22 ± 0.23	1.16 ± 0.32	1.32 ± 0.32
Alkaline phosphatase (KAU)	7.9 ± 3.0 ^c	6.4 ± 1.8 ^c	8.4 ± 3.4 ^c	6.4 ± 2.3 ^c	14.0 ± 10.0 ^b	19.9 ± 15.3 ^a

¹ $\bar{x} \pm \text{SD}$. 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)₂D, 1,25-dihydroxyvitamin D; PTH-i, intact parathyroid hormone; KAU, King-Armstrong units; ND, not done. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

Direct sunlight exposure was assessed by documenting average duration of exposure and percentage of the surface area of the body exposed daily (11). The average duration of cloud-free sunshine during the year of this study was 3.1 h/d in the winter (December to February 1997–1998) and 7.1 h/d in the summer (April to July 1998) in Delhi [28.35°N and 77.12°E with zenith angle of 84.5° in peak summer and 38.5° in peak winter (Meteorology Department, Delhi)]. The ground surface of Delhi receives 4 MED (minimal erythemal dose) of ultraviolet radiation/d during the summer and only 1 MED/d in the winter (10). The skin pigmentation of the subjects was classified visually as dark, wheatish, or fair. In all groups except the depigmented group, the proportion of subjects in each pigment category was not significantly different, with >60% of subjects in each group having a wheatish complexion. The depigmented group was studied in reference to the other groups to evaluate the effect of skin pigmentation.

Nutritional status was assessed by estimating the average composition of the daily diet in terms of energy, carbohydrate, protein, fat, calcium, and phytate (phytin-P) by use of a semi-quantitative food-frequency questionnaire (12) and published data on the nutrient composition of Indian food (13). Dairy products and food fats are not fortified with vitamin D in India.

Blood samples were collected without venostasis from the different groups under basal conditions and cord blood was collected on ice from the newborns for estimations of serum 25(OH)D, 1,25(OH)₂D, PTH-i, total calcium, inorganic phosphate, and alkaline phosphatase activity. The serum was separated in a refrigerated centrifuge at 1200 × g for 15 min at 4°C, aliquoted, and stored at –20°C until analyzed. Serum PTH-i could be measured only in groups studied in the winter. Serum total calcium, inorganic phosphate, and alkaline phosphatase were measured by methods described previously (8). Serum samples from pregnant women group were heated at 65°C for 30 min before alkaline phosphatase estimation to exclude placental isoenzyme activity (14). Serum 25(OH)D [reference range (2 SDs): 22.2–116.5 nmol/L] and PTH-i [reference range (2 SDs): 13–54 ng/L] were measured by radioimmunoassay and immunoradiometric assay, respectively (catalog no. 68100 and

26100; Incstar Corporation, Stillwater, MN). 1,25(OH)₂D concentrations were estimated by Quest Diagnostics (Nichols Institute, San Juan Capistrano, CA). Briefly, the assay involved extraction with hexane-isopropanol followed by Sep-Pak C₁₈ purification (Millipore, Milford, MA). Quantification was done by adopting a nonequilibrium radiobinding assay by using 1,25(OH)₂D receptors from calf thymus (15). The normal reference range, sensitivity, and intra- and interassay variations were 36–144 pmol/L, 4.8 pmol/L, and 8% and 13%, respectively. The 1,25(OH)₂D assay could be performed only in a representative subset of 19 subjects each in the 3 groups studied in the summer.

Data are presented as means ± 1 SD. One-way analysis of variance was used to establish whether differences existed within study groups. If a significant difference was found, a multiple comparison test was performed with a Bonferroni post hoc test to analyze differences between the study groups. P values <0.05 were considered significant. The paired t test was used to compare differences in indexes between summer and winter in the physician and nurse group. Pearson and Spearman rank tests were used for correlation analysis as appropriate.

RESULTS

The study groups' mean ages, sunlight exposure, and serum values of 25(OH)D, 1,25(OH)₂D, PTH-i, total calcium, inorganic phosphate, and alkaline phosphatase are summarized in **Table 1**. The physician and nurse group and the soldier group were normal nutritionally in terms of their body mass indexes. The mean daily dietary energy, carbohydrate, fat, and protein intakes of the physician and nurse and soldier groups, respectively, were 8217 ± 1857 and 14875 ± 2363 kJ, 297 ± 60 and 478 ± 114 g, 60 ± 16 and 85 ± 25 g, and 59 ± 13 and 100 ± 28 g. The dietary energy, carbohydrate, fat, and protein intakes of these 2 groups were normal according to Indian normative data published by the Indian Council of Medical Research (ICMR) (12). All the groups consumed predominantly vegetarian diets and ate meat only 2–3 times/wk. Fish was a rare diet item. The mean dietary calcium contents of the physician and nurse group and the soldier group were not significantly different. There



was no significant difference in dietary composition in the physician and nurse group between winter and summer. The daily energy, carbohydrate, fat, and protein intakes of the depigmented group were 8055 ± 1727 kJ, 277 ± 59 g, 65 ± 22 g, and 58 ± 20 g, respectively, and were also in the normal range prescribed by the ICMR.

The diet of the pregnant group was deficient in energy (6903 ± 1673 kJ) and protein (40 ± 10 g) with reference to the ICMR recommendations of 17043 kJ and 65 g protein daily (13). The mean carbohydrate and fat contents of the pregnant group's diet were 268 ± 88 and 45 ± 14 g, which were significantly less than the corresponding intakes of the soldier group. The mean calcium content of the pregnant group's diet was also significantly less than that of the soldier group's and was far less than the ICMR recommended daily allowance for pregnant women in India (1000 mg).

The mean ratio of phytate to calcium in the daily diet of the physician and nurse, soldier, and depigmented groups was not significantly different. However, the pregnant group had a significantly higher ratio than did all other groups.

In the winter, the mean direct sunlight exposure of the soldier group was significantly higher (because of daily outdoor physical training) than that of the physician and nurse group. The depigmented group (the other group studied in the winter) had negligible sunlight exposure (5 ± 5 min) by preference. The average body surface area exposed in all 3 groups studied in the winter was 10% (face and hands only). In the summer, the mean duration of direct sunlight exposure in the physician and nurse and pregnant groups was 25 ± 5 min. However, the skin surface area exposed to direct sunlight during this time was 20% as a result of summer dress.

Mean 25(OH)D concentrations varied among the different study groups and were related to direct sunlight exposure and skin pigmentation. The highest mean 25(OH)D concentration was measured in the soldier group. The physician and nurse group, which had significantly less sunlight exposure than did the soldier group, also had significantly lower 25(OH)D concentrations. Interestingly, the depigmented group, for whom sunlight exposure was significantly lower than for the physician and nurse group in the winter, had significantly higher 25(OH)D values than in the physician and nurse group, presumably because of absent skin pigmentation. However, 25(OH)D concentrations in both the physician and nurse and depigmented groups were significantly lower than those in the soldier group and far below the mean values reported in healthy whites with use of the same kit.

When restudied in the summer, the physician and nurse group had significantly higher 25(OH)D concentrations than in the winter. There was no significant difference in mean 25(OH)D between the pregnant and the physician and nurse groups in the summer. The mean cord blood 25(OH)D concentration of the newborns was significantly lower than that of the pregnant group. Correlative analysis of 25(OH)D values in pregnant women and their neonates showed a significant positive correlation between the 2 variables ($r = 0.7934$, $P < 0.001$).

In the winter, the mean serum total calcium concentration measured in the soldier and depigmented groups was significantly higher than that in the physician and nurse group. The corresponding mean values of PTH-i were significantly lower in the soldier group than in the physician and nurse and depigmented groups. There was no significant difference in mean inorganic phosphate and alkaline phosphatase values among the

3 groups studied in the winter. The mean serum total calcium concentration measured in the physician and nurse group in the summer tended to be higher than the mean measured in the same group in the winter. Serum inorganic phosphate and alkaline phosphatase values did not differ significantly between winter and summer.

In the summer, the mean serum total calcium concentration (adjusted for serum albumin) measured in pregnant women was significantly lower than that in the physician and nurse group. This was associated with significantly higher mean serum alkaline phosphatase values in the pregnant group than in the physician and nurse group. The mean serum total calcium concentration in cord blood of newborns was significantly higher than maternal serum total calcium. There was a significant positive correlation between maternal and cord blood total calcium values ($r = 0.5129$, $P < 0.05$). There was no significant difference in serum inorganic phosphate between the 3 groups studied in the summer.

In the summer, the physician and nurse and newborn groups had significantly lower mean $1,25(\text{OH})_2\text{D}$ concentrations than did the pregnant group. Correlative analysis showed a positive correlation between cord blood and maternal $1,25(\text{OH})_2\text{D}$ values ($r = 0.4527$, $P = 0.059$).

When the serum 25(OH)D and calcium values of all the subjects studied in the different groups, in both summer and winter, were pooled and analyzed, a significant direct relation was found between the 2 variables ($r = 0.1769$, $P = 0.036$). When PTH-i and 25(OH)D values measured in all 3 groups studied in the winter were pooled, a significant inverse relation was found between 25(OH)D and PTH-i ($r = -0.6303$, $P < 0.001$). Analysis of the PTH-i and serum total calcium values obtained from all 3 groups studied during the winter showed a significant inverse relation between the 2 variables ($r = -0.4281$, $P < 0.01$).

DISCUSSION

The reported paradox of the prevalence of rickets and osteomalacia in the sun-drenched South Asian countries remains unexplained. Recent reports of low 25(OH)D concentrations in healthy subjects resident in South Asia (8, 9) and elsewhere in the tropics (16, 17) question the functional relevance of 25(OH)D measurement. The present study addressed some of these issues. We can draw the following conclusions from our comparative analysis of 25(OH)D and related bone mineral metabolic variables in the groups of healthy subjects:


- 1) Although dietary intakes of calcium and phytate were not significantly different, the soldier group had significantly higher serum 25(OH)D and total calcium and significantly lower PTH-i concentrations than did the physician and nurse group in the winter. This finding may be explained by the significantly longer duration of direct sunlight exposure in the soldier group.
- 2) Compared with the physician and nurse group, the depigmented group had significantly higher 25(OH)D concentrations despite a shorter duration of direct sunlight exposure. This observation emphasizes the relevance of pigmentation in retarding sunlight-mediated dermal vitamin D synthesis (4, 7, 18). However, despite the advantage of absent pigmentation, the depigmented group was deficient in 25(OH)D with reference to the mean and range reported in whites with use of the same assay kit. Matsuoka et al (19) reported lesser efficiency



of dermal vitamin D synthesis in the head and neck and upper arm regions than in the trunk and lower limbs in whites. The present group of depigmented subjects studied in the winter wore clothing that permitted sunlight exposure of only the head and neck region and hands. The significantly lower duration of direct sunlight exposure in the depigmented subjects may also have contributed to their deficient vitamin D status.

- 3) The relative normalization of serum calcium in the physician and nurse group was attained through a PTH-mediated resorptive process of bone mineral. This becomes evident when the significantly lower serum total calcium and significantly higher PTH-i concentrations in the physician and nurse group in the winter is viewed in the context of this group having the lowest mean value of 25(OH)D and all the groups studied in the winter showing an inverse relation between serum 25(OH)D and total calcium on one hand and PTH-i on the other. Such chronic and insidious PTH-dependent bone resorption is known to be relevant in the pathogenesis of osteoporosis (20). On the basis of physical determination of the porosity of bones obtained at autopsy from accidental death victims, Ahuja (21) documented significantly lower bone density in healthy subjects from Delhi than that reported in developed countries. The relative deficiency of vitamin D in Delhi with seasonally varying and chronically increased PTH activity may be causally linked to such relative osteopenia among healthy Indians (21).
- 4) When restudied in the summer, the physician and nurse group had improved yet still subnormal 25(OH)D concentrations; there were no seasonal differences of significance in serum total calcium, inorganic phosphate, and alkaline phosphatase activity. The pregnant group, which was also studied in the summer, had significant chemical osteomalacia even though mean 25(OH)D concentrations were not significantly different from those in the physician and nurse group.

Did the deficient calcium and high phytate content of the pregnant group's diet worsen this group's vitamin D status through the mechanism proposed by Fraser et al (22)? In view of the deficient 25(OH)D concentrations measured in both the pregnant and the physician and nurse groups in the summer, it cannot be propounded that the rate of hepatic metabolism of 25(OH)D was increased in the pregnant group as a result of increased 1,25(OH)₂D generation. A more plausible explanation is that, in the presence of deficient 25(OH)D, chemical osteomalacia occurred in the pregnant group as a result of this group's high calcium requirement and deficient intake coupled with a high dietary ratio of phytate to calcium. The relatively high circulating 1,25(OH)₂D concentrations would not optimize calcium absorption in this group.

In summary, despite abundant sunlight, healthy persons in Delhi remain vitamin D deficient because of skin pigmentation and inadequate direct sunlight exposure. When exposed to factors that adversely affect vitamin D and bone mineral metabolic status, an imbalance in bone mineral metabolic homeostasis results. Among such factors, low-calcium, high-phytate diets; pregnancy; and winter-related reduced sunlight exposure are important. 

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REFERENCES

1. Hodgkin P, Kay GH, Hine PM, Lumb GA, Stanbury SW. Vitamin D deficiency in Asians at home and in Britain. *Lancet* 1973;2:167-72.
2. Deluca HF, Krisinger J, Darwish H. The vitamin D system: 1990. *Kidney Int* 1990;38(suppl):S2-8.
3. Holick MF, Maclaughlin JA, Clark MB, et al. Photosynthesis of pre-vitamin D₃ in human skin and the physiologic consequences. *Science* 1980;210:203-5.
4. Clemens TL, Adams JS, Henderson SL, Holick MF. Increased skin pigmentation reduces capacity of the skin to synthesise vitamin D₃. *Lancet* 1982;1:74-6.
5. Hollis BW, Napoli LJ. Improved radioimmunoassay for vitamin-D and its use in assessing vitamin D status. *Clin Chem* 1985;31:1815-9.
6. Bhattacharya AK. Nutritional rickets in the tropics. *World Rev Nutr Diet* 1992;67:140-97.
7. Lo CW, Paris PW, Holick MF. Indian and Pakistani immigrants have the same capacity as Caucasians to produce vitamin D in response to ultraviolet irradiation. *Am J Clin Nutr* 1986;44:683-5.
8. Harinarayan CV, Gupta N, Kochupillai N. Vitamin D status in primary hyperparathyroidism. *Clin Endocrinol (Oxf)* 1995;43:351-8.
9. Brunvand L, Shah SS, Bergstrom S, Haug E. Vitamin D deficiency in pregnancy is not associated with obstructed labor. A study among Pakistani women in Karachi. *Acta Obstet Gynecol Scand* 1998;77:303-6.
10. Sharma MC, Jain VC, Srivastava BN. Calculation of erythematous doses at Indian subcontinent. *Proc Indian Natl Sci Acad* 1982;48:72-82.
11. Mcgrouter DA. Skin burns. In: Mann CV, Russel RCG, Williams NS, eds. *Bailey and Love's short practice of surgery*. London: Chapman and Hall, 1995:124-48.
12. Willet WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a food frequency questionnaire. *Am J Epidemiol* 1985;122:51-65.
13. Food composition tables. In: Gopalan C, Sastri BVR, Balasubramanian SC, eds. *Nutritive value of Indian foods*. Hyderabad, India: National Institute of Nutrition, Indian Council of Medical Research, 1996:45-95.
14. Moss DW, Henderson AR. Clinical enzymology. In: Burtis CA, Ashwood ER, eds. *Teitz text book of clinical chemistry*. Philadelphia: WB Saunders, 1999:617-721.
15. Reinhardt TA, Horst RL, Orf JW, Hollis BW. A microassay for 1,25-dihydroxyvitamin D not requiring high performance liquid chromatography: application to clinical studies. *J Clin Endocrinol Metab* 1984;58:91-98.
16. Sedrani SH, Eldrissy AW, El Arabi KM. Sunlight and vitamin D status in normal Saudi subjects. *Am J Clin Nutr* 1993;38:129-32.
17. Markestad T, Elzouki A, Legnain M, Ulstein M, Aksnes L. Serum concentrations of vitamin D metabolites in maternal and umbilical cord blood of Libyan and Norwegian women. *Hum Nutr Clin Nutr* 1984;38:55-62.
18. Matsuoka LY, Wortsman J, Hadad JG, Kolm P, Hollis BW. Racial pigmentation and the cutaneous synthesis of vitamin D. *Arch Dermatol* 1991;127:536-8.
19. Matsuoka LY, Wortsman J, Hollis BW. Use of topical sunscreen for the evaluation of regional synthesis of vitamin D₃. *J Am Acad Dermatol* 1990;22:772-5.
20. Chapuy MC, Arlot ME, Duboeuf F, et al. Vitamin D₃ and calcium to prevent hip fractures in elderly women. *N Engl J Med* 1992;327:1637-42.
21. Ahuja M. Normal variation in the density of selected human bones in North India. *J Bone Joint Surg Br* 1969;51B:719-35.
22. Clements MR, Johnson L, Fraser DR. A new mechanism for induced vitamin D deficiency in calcium deprivation. *Nature* 1987;325:62-5.

