

**ORIGINAL****Impact of dietary and lifestyle on vitamin D in healthy student girls aged 11-15 years**

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**Abstract : Objective :** To study daily intake of calcium, phosphorus and vitamin D, to determine the biochemical findings of rickets and the effect of sunlight exposure and vitamin D supplementation in school girls with hypovitaminosis D.

**Methods:** A cross-sectional study was conducted on school girls aged 11-15 years selected randomly from various areas of Tehran, Iran. Dietary information and amount of sunlight exposure were estimated by a 7 day recalling method using self-reported questionnaire. Hypovitaminosis D defined as low serum 25-hydroxyvitamin D concentration with two or more others abnormal biochemical findings. Girls with hypovitaminoses D were randomly divided into two groups. The faces and hands of girls in group 1 were exposed to sunlight for one hour per day for twenty days, while those in group 2 were administered vitamin D capsules, 50,000 IU per day for the same period.

**Results :** four-hundred fourteen girls evaluated, mean daily calcium intake, sunlight exposure and vitamin D acquirement were 360 mg, 10 minutes and 119 IU, respectively. Mean serum 25-hydroxyvitamin D concentration was 30 ng/ml among all girls whereas in 15 (3.63%) of 414 girls was 7.8 ng/ml. Abnormal biochemical findings in these girls included hypocalcemia (n=4), hypophosphatemia (n=5), raised serum alkaline phosphatase (n=13), and parathyroid hormone (n=15). After intervention, mean serum 25-hydroxyvitamin D concentration in sunlight exposure (n=8) and vitamin D (n=7) supplementation increased to  $14.4 \pm 4$  ng/ml and  $23 \pm 4$  ng/ml respectively. There was a significant difference between the two groups ( $p < 0.05$ ).

**Conclusion:** Vitamin D deficiency developed in rapid growth period of girls without clear clinical rickets in sunny temperate climate city in Iran which vitamin D supplementation improved biochemical findings better than sunlight exposure. *J. Med. Invest.* 53:204-208, August, 2006

**Keywords :** 25-hydroxyvitamin D, intake, calcium, sun exposure

**INTRODUCTION**

Although deficiency of either calcium or vitamin D can cause nutritional rickets. Vitamin D deficiency is attributed to a variety of causes including diet, atmospheric pollution, religious practices that

restrict sunlight exposure (clothing), geographic latitude and altitude, season, time of the day (1-4). Tehran is located at latitude  $35^\circ$  with temperate climate. The dress code for Islamic Iranian women and adolescent girls is total coverage of body with long garments and Maghnaeh (Islamic scarf). The aim was to report abnormal biochemical results of rickets in apparently healthy adolescent girls living in a sunny environment and therapeutic intervention with sunlight exposure and vitamin D supple-

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mentation in the girls with abnormal results and will discuss the risk factors for development of rickets. This research project approved by research and ethic committee of Shaheed Beheshti Medical University.

## METHODS

A randomized, cluster sample of girls, cross-sectional, prospective and descriptive study was carried out from January to March 2003 for the estimation of daily intake of calcium, phosphorus, vitamin D and also the detection of serum biochemical findings of rickets. After a brief explanation about causes, symptoms, morbidity and method of treatment of rickets for parents by letter and obtaining the informed consent from one of the parents the girl included in the study. The girls were requested to report their parental education and employments, number of families, type of house, ordinary daily food intake, use of calcium or vitamin D supplementation, daily use of soft drink and milk, the average daily duration of sunlight exposure on their faces and hands during school term, holidays and physical activities in regard to the hours spent on school exercise and leisure activities. The mean daily dietary intake of calcium, phosphorus and vitamin D were estimated by seven day foods recall method using self-reported questionnaire. A nutritionist checked each questionnaire and approximately calculated daily intake of calcium, phosphorus, vitamin D and skin sunlight exposure (uncovered face and hands) for vitamin D synthesis. Weight, height, Tanner stages of all girls recorded on their questionnaires.

Physical examination and taking previous history of the diseases were conducted by two pediatricians. The girls were excluded with renal, hepatic or bone disease, malabsorption, anticonvulsant therapy and supplementary vitamin D and calcium. No paraclinical studies were done to rule out chronic disease. Fasting blood samples were collected by venipuncture. After blood centrifugation, serum was separated and stored at -20°C until analysis of 25-hydroxyvitamin D [25-(OH) D] and parathyroid hormone (PTH) by radioimmunoassay (Gamma counter system, genesys). Routine blood chemistry included serum calcium, phosphorus and alkaline phosphatase (ALP) were analyzed by Hitachi 717 system, autoanalyzer RXT technicon. The normal serum laboratory reference

range of biochemical findings include 25-(OH)D= 7.6-75 ng/ml, PTH=16-62 pg/ml, ALP=170-1000 IU/l, calcium=8.6-10 mg% and phosphorus=2.5-5 mg%.

On the basis of results, the hypovitaminosis D was defined as serum 25-(OH) D less than 7.6 ng/ml with two or more abnormal biochemical findings of rickets such as low serum phosphorus, low or normal calcium and raised serum PTH or ALP concentration. The girls with abnormal biochemical findings in a randomized trial divided into two groups. Group I, their hands and faces were exposed to sunlight for one hour/day for 20 days and group II, administered 50,000 IU vitamin D capsules/day (Zahravi, Tabriz-Iran) for 20 days.

The serum of all girls were collected for measurement of calcium, phosphorus, ALP, PTH, and 25-(OH)D one day after the end of intervention. Statistical analysis was conducted by SPSS 11.5. Statistical software were used to run the statistical analysis. The results were expressed as mean  $\pm$  standard deviation (S.D). The significance level was set at  $P < 0.05$ . Comparisons of means among groups were done with two-sample t-tests.

## SUBJECTS

Consisting healthy adolescent middle school student girls 11-15 years old from various areas of Tehran, Iran.

## RESULTS

The study group comprised of 414 apparently healthy girls aged 11-15 years with various socioeconomic backgrounds (based on their fathers' employment). The daily vitamin D acquirement of all girls was below the recommended daily allowance of 400 IU. The mean daily dietary intake of calcium, phosphorus, vitamin D and characteristics of the girls are shown in Table I.

Dietary evaluation revealed that bread is the main diet and then cereal, rice, meat, vegetables. Milk and milk products were not as an important source of dietary calcium intake. The milk is not fortified with vitamin D, and it is used infrequently and in low quantities. Nobody was vegetarian. Approximately, 32 (8%) received daily dietary calcium intake above 1200 mg that is more than recommended daily allowance for these age groups.

None of the girls were receiving vitamin D and calcium supplementation. There were no significant differences in daily calcium intake among the serum normal and abnormal findings of the girls. Fifteen (3.63%) of the 414 girls were hypovitaminosis D. Their characteristics are shown in Table 1 .

Table II shows the biochemical findings of the girls before and after intervention which have improved all results. Serum 25-(OH) D concentration in group II is significantly higher than group I.  $p < 0.05$ .

## DISCUSSION

Table I . Characteristics of the girls in the cross-sectional survey

Variable	Mean values $\pm$ SD (range)		
	Total	Group I	Group II
Number	414	8	7
Age (year)	13 $\pm$ 1 ((11-15)	13 $\pm$ 1 (11-15)	13 $\pm$ 1 (11-15)
Weight (kg)	47 $\pm$ 11 (27.5-77.5)	43.6 $\pm$ 39 (31-65)	44 $\pm$ 9 (30-69)
Height (cm)	147 $\pm$ 7 (130-160)	152 $\pm$ 15 (108-170)	155 $\pm$ 8 (134-174)
Tanner stage	3 $\pm$ 1 (1-5)	3 $\pm$ 1 (1-5)	3 $\pm$ 1 (1-5)
Dietary intake			
Vitamin D IU/d	119 $\pm$ 52 (12.5-312.5)	110 $\pm$ 53 (30-240)	124 $\pm$ 45 (40-200)
Calcium mg/d	360 $\pm$ 350 (33-2623)	471 $\pm$ 383 (72-1447)	621 $\pm$ 490 (206-1381)
Phosphorus mg/d	1137 $\pm$ 550 (108-4500)	985 $\pm$ 438 (568-1250)	1050 $\pm$ 397 (630-2184)
Exercise min/d	10 $\pm$ 5 (10-30)	10 $\pm$ 7 (10-35)	10 $\pm$ 5 (10-30)
Sunlight exposure min/d	10 $\pm$ 18 (5-40)	10 $\pm$ 8 (5-25)	10 $\pm$ 7 (5-30)

Table II . Biochemical findings of rickets in two groups before and after intervention

Serum parameter	group I (sunlight exposure)			group II (vitamin D intake)		
	before	n = 8	after	before	n = 7	after
Ca mg%	8.34(1.33)		9.02(0.67)	8.7(0.85)		9.7(0.08)
P mg%	3.4(0.97)		4(0.6)	3.3(1.2)		4.4(1)
ALP IU/l	1194 (525)		565 (226)	1381(383)		617(295)
PTH pg/ml	133 (81.78)		28(10)	104 (27)		22(4.5)
25-(OH)D ng/ml	7 (2)		14(4)	7.5(2)		23(4)

Values expressed as mean ( $\pm$ SD).

Nutritional rickets may be caused by inadequate vitamin D or calcium intake, especially during rapid growth phases. Vitamin D is essential for the maintenance of calcium homeostasis and bone mineralization. Under normal condition the endogenous synthesis of vitamin D is more important than that obtained by dietary intake (5-7).

Assessment of dietary intake or acquirement of vitamin D by sunlight exposure and calcium intake by a self - reported questionnaire has limitation of accuracy and assessment of detailed quantitative dietary nutrients could not be calculated. However in our situations it was the best method because home and school visits to assess dietary

intake of calcium or vitamin D and sunlight exposure would have been culturally impossible. As we previously showed, the low serum 25-(OH) D concentration in all fifteen girls may be due to low skin exposure to ultraviolet radiation and lifestyle such as total coverage of body with long garments and Maghnaeh or unfortified food intake in most instances and skin pigmentation, season, atmospheric pollution in rare instances. Low vitamin D may be associated with decreased calcium absorption and failure of the osteoid to mineralize, and this may ultimately lead to overt clinical rickets(2-3). As a result of vitamin D deficiency, calcium absorption will be reduced and PTH concentration will rise to maintain plasma calcium at a physi-

ologically optimum level by inducing calcium mobilization from the bone(2-4). Subsequently, secondary hyperparathyroidism will occur. This study demonstrated secondary hyperparathyroidism in all girls. Although, administration of vitamin D and sunlight exposure improved all abnormal biochemical findings of two groups but vitamin D concentration in group I is lower than group II ( $P < 0.05$ ) and we suppose that daily duration of sunlight exposure was low. The serum concentration of 25-(OH) D is the most sensitive biochemical marker of subject's vitamin status(8). The present study demonstrated the prevalence rate of hypovitaminosis D in 15 (3.63%) girls, whereas the mean daily acquirement of vitamin D in all girls were approximately less than 120 IU/d. The explanation of these differences may be due to various eating habits and lifestyle among normal and abnormal groups.

Other factors of vitamin D deficiency that can explain in these groups include association between iron deficiency and vitamin D deficiency that has been described in the UK in Asian pre-school children(9) that may be due to dietary and lifestyle factors such as rapid growth and onset of menstruation that lead to deficiency in both nutrients. The beneficial effects of iron supplementation on vitamin D status has been described and suggest that iron deficiency affects gut absorption of vitamin D(10). However, we did not measure serum iron in these girls but as we have shown, it can be due to lifestyle and nutrient factors. The other factors that contribute to the vitamin D deficiency are unfortified milk or other nutrients with vitamin D that these girls consume.

The main dietary factor that has been associated with vitamin D deficiency is bread. It has been postulated that the high phytate content of bread may interfere with enterohepatic circulation of vitamin D metabolites(11). In one adult study, high phytate consumption accounted for 12% of the variance in vitamin D level(12) and was a significant risk factor for rickets in the UK(13-14). The mean vitamin D intake is significantly lower in central Europe than Northern America(15) that is similar in our current study. Utiger(16) suggest that vitamin D supplementation should be used more widely, and also peripubertal children should consume daily vitamin D supplementation. In countries such as Iran low exposure to sunlight and reduced synthesis of vitamin D have also been implicated in rickets and in current study we

demonstrated hypovitaminosis D in rapid growth period of adolescent girls.

## CONCLUSION

The current study demonstrated biochemical findings of rickets due to vitamin D deficiency in a sunny temperate climate city during rapid growth phase in student girls without clear clinical rickets. Vitamin D supplementation intake improved biochemical findings of rickets better than sunlight exposure. The prevention of progressive damage and morbidity rates depend on measures to encourage them to increase sunlight exposure by a sequential program or vitamin D should be supplemented in their nutrient foods.

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## REFERENCES

1. Bhattacharyya AK : Nutritional rickets in the tropics. *World Rev Nutr Diet* 67 : 140-97, 1992
2. Holick MF, Maclaughlin JA, Clark MB, Holick SA, Potts J T, Anderson RR, Blank IH, Parrish JA, Elias P : Photosynthesis of previtamin D 3 in human skin and the physiologic consequences. *Science* 210 : 203-5, 1980
3. Holmberg I, Larsson A : Seasonal variation of vitamin D 3 and 25-hydroxyvitamin D 3 in human serum. *Clin Chim Acta* 100 : 173 -174, 1980
4. Hutchinson G, Hall A : The transmission of ultraviolet light through fabrics and its potential role in the cutaneous synthesis of vitamin D. *Hum Nutr Appl Nutr* 38A : 298-302, 1984
5. Moncrieff MW, Lunt HR, Arthur LJ : Nutritional rickets at puberty. *Arch Dis Child* 48 : 221-4, 1973
6. Lawson DEM, Poul AA, Black A, Cole TJ, Mandal AR, Davie M : Relative contributions

- of diet and sunlight to vitamin D state in the elderly. *Br Med J* 2 : 303-5, 1979
7. Lawson DEM : Dietary vitamin D : is it necessary? *J Hum Nutr* 35 : 61-63, 1981
  8. Hollis BW : Assessment of vitamin D nutritional and hormonal status : what is measure and how to do it. *Calcif Tissue Int* 54 : 4-5, 1996
  9. Grindulis H, Scott PH, Belton NR, Wharton BA : Combined deficiency of iron and vitamin D in Asian toddlers. *Arch Dis Chil* 61 : 843-48, 1986
  10. Heldenberg D, Tenenbaum G, weismany : Effect of iron on serum 25 - hydroxyvitamin D and 24, 25 dihydroxy vitamin D concentrations, *Am J Clin Nutr* 56 : 553-36, 1992
  11. Clements MR : The problem of rickets in UK Asian. *J Hum Nutr Dieter* 2 : 105-16, 1989
  12. Hunt SP, Nash AH, Watson R, Truswells : Vitamin D status in different subgroups of British Asian. *BMJ* 1 : 641, 1977
  13. Dunnigan M, Mcintosh W, Ford J : Ricket in Asian immigrants. *Lancet* 1 : 1346, 1976
  14. Ellis G, Wood Bead J, Cooke W : Serum 25-hydroxyvitamin D concentrations in adolescent boys. *Lancet* 1 : 823-8, 1977
  15. Mckenna MJ : Differences in vitamin D status between countries in young adults and elderly. *Am J Med* 93 : 69-77, 1992
  16. Utiger RD : The need for more vitamin D. *N Engl J Med* 338 : 828-9, 1998