

The Influence of Sunlight Exposure on Serum Vitamin D Concentration and Bone Turnover; a controlled clinical trial

*A Ataie-Jafari, A Hossein-nezhad, Zh Maghbooli, F Karimi, M Rahmani, S Shahbazi,*B Larijani*

Endocrinology and Metabolism Research Center of Tehran University of Medical Sciences, Iran

Abstract

Background: Sunlight exposure is one of the ways for vitamin D synthesis. However, its effect on vitamin D status via experimental studies is poorly understood. This study was undertaken to address the possibility that sunlight exposure may increase the levels of serum vitamin D, and alter bone turnover in healthy young girls.

Methods: In a controlled clinical trial, young girls were assigned to the test group (n= 45) or control group (n= 80). An outdoor swimming pool was considered for this project and the test group was required to participate in these sessions at least for 8 sessions and to expose to direct sunlight at least for 20 minutes in each session. They were not allowed to use sun-screen during this time. Control group continued their usual manner of sun exposing. Serum levels of vitamin D, calcium, alkaline phosphatase, parathormone, osteocalcin and crossLaps were measured before and after duration of the study in both groups and compared between them.

Results: Subjects aged 27.46 ± 8.78 years. Serum levels of vitamin D and bone markers were constant during the study in both groups. Changes of these variables were not significant between the groups after the study. Serum vitamin D in subjects with white skin color correlated with total time of direct sun exposing after the study ($P= 0.002$).

Conclusion: Sunlight exposure did not affect the serum vitamin D and bone turnover in healthy young girls. However, subjects with bright skin complexion benefit from sunlight exposing more than those with a dark skin color in the case of vitamin D improvement.

Keywords: *Sunlight exposure, Vitamin D, Bone turnover*

Introduction

Vitamin D deficiency is a common health problem in many countries (1-3). This problem is associated with an increased risk of osteoporosis and other chronic diseases such as cancers, autoimmune diseases and cardiovascular disease (4-6).

Vitamin D is supplied by consumption of vitamin D-rich foods and by vitamin D synthesis in skin. Unfortunately, a few foods naturally contain vitamin D, and only a few foods are fortified with vitamin D. This is the reason why vitamin D deficiency has become prevalent for all age groups in the United States and Europe (4). The major source of vitamin D is UV light exposure and anything that influences the amount of light reaching the deeper epidermal layers of the skin, such as season, latitude, clothing, and darker skin color, can influence vitamin D status (4). Most humans depend on sun exposure

to satisfy their requirements for vitamin D. Solar ultraviolet B photons are absorbed by 7-dehydrocholesterol in the skin, leading to its transformation to previtamin D₃, which is converted to vitamin D₃. Once formed, vitamin D₃ is metabolized in the liver to 25-hydroxyvitamin D₃ and then in the kidney to 1, 25-dihydroxyvitamin D₃ (4).

According to the information mentioned above, it could be logical to suggest low prevalence of vitamin D deficiency in tropical countries. However the studies carried out in the previous two decades have shown a high prevalence of vitamin D deficiency in tropical countries such as India (7), Turkey (8) and Saudi Arabia (9).

In a study in Turkey (8), serum 25(OH) D levels were compared in 48 women with different types of dressing in summer. Results showed a higher level of serum vitamin D in women dressed in a style which exposed the usual areas

of the skin to sunlight than women dressed in traditional Islamic style and covering the whole body including hands and face.

The study in a population from IMOS (the Iranian Multicenter Osteoporosis Study) revealed a high prevalence of vitamin D deficiency in Tehran, Iran. However, serum vitamin D levels had no significant statistical relation with the duration of exposure to sunlight and kind of clothing (10).

A sensible sun exposure (usually 5–10 min of exposure of the arms and legs or the hands, arms, and face, 2 or 3 times per week) and increased dietary and supplemental vitamin D intakes are reasonable approaches to guarantee vitamin D sufficiency (4).

To determine changes of bone metabolism and its relation with vitamin D, measuring bone turnover markers is suggested. Whereas in a study on a young adult healthy Lebanese population, there was a significant negative correlation between urinary-free deoxypyridinoline (DPD) and 25(OH)D as well as a significant positive correlation between parathormone (PTH) and free DPD and between PTH and osteocalcin. A positive correlation was also found between 25(OH) D and osteocalcin (11).

Since there is no clinical trial addressing the possible influence of direct effect of sunlight exposure on serum vitamin D and bone turnover in healthy population, we carried out this controlled study in a group of healthy young girls to assess this effect while a large area of the skin uncovered and exposed to the sunlight.

Material and Methods

Subjects

The volunteers were recruited from girl students between 18-35 yr old after an announcement in Tehran University of Medical Sciences (TUMS) and dormitory. Subjects with menarche disorders, spinal deformity and using estrogen, progesterone, anti-convulsion drugs, rheumatoid arthritis, hyper or hypothyroidism, parathyroid or adrenal problems, diabetes, renal failure, liver diseases or every kind of cancer were ex-

cluded from the study. We excluded subjects who reported using any kind of vitamin D supplements or multivitamins containing vitamin D or fish oil supplements during the last 3 months.

The study was approved by Ethical Committee of Endocrinology & Metabolism Research Center of TUMS and all subjects gave informed consent to participate.

Study Design

This was a controlled clinical trial which was performed during the 2 warmest months of summer. The study was carried-out in Tehran located between latitude 34° 52' toward 36° 21' N and longitude 50° 10' towards 53° 10' E with an altitude of 1190 meter from the surface of the sea. The trial was performed during July-August with the average temperature of 30.5° centigrade. The mean of sunny hours of Tehran is 362 h in July and 358 h in August (13).

The goal of study was explained for participants and they were allocated to one of the groups as control or test. We considered an outdoor swimming pool for this project and the test group was required to participate in these sessions at least for 8 sessions and to expose to direct sunlight at least for 20 min in each session. They were not allowed to use sunscreen or rub any substance on their skin during this time. Control group continued their usual manner of sun exposing during the study. All subjects were advised not to change their eating habits during the whole study and were checked every week by telephone.

The volunteers provided a 38-item FFQ which was mainly designed for calcium and vitamin D intake assessment. Frequencies of food intakes during past month were reported in day, week, or month. Calcium content of foods was derived from Nutritionist III software modified for Iranian foods and vitamin D was calculated according to the table of the vitamin D content of foods (12). Weight and standing height were measured without shoes and body mass index (BMI; in kg/m²) was calculated.

The usual time per day spent outdoors in daylight hours during the previous month was re-

ported as <15 min, 15-30 min and 30-60 min, and the extension of sunlight exposure reported as "limited to hand and face", or "more than hand and face". Protection of skin with the use of sunscreen was reported as "yes", "sometimes", and "never". Participants reported the kind and the average time per day that they spent on physical activity.

In the beginning of the study, the color of skin (inner arm) was recorded as white, swarthy, and brown in both groups. The test group was provided with a questionnaire in which they were required to register the number of sessions they participated in swimming-pool and the duration they exposed to direct sunlight. These two variables were multiplied and total time (in minutes) of sun exposure for each person in sunlight-exposed group was calculated by summing up these figures. Sunlight intensity was recorded every two hours during the times that the test group were advised to expose it. Photometry was done (Lunasix, Germany) by one of the authors. The degree of skin color changes in the test group were recorded as "low change", "medium change", "high change" and "very high change" at the end of the study.

Laboratory Measurements

Blood samples were drawn in the morning after 10-12 h overnight fasting by vacuator tubes (Vacurette, Greiner Bio-One GmbH, Gapan) allowed to clot at room temperature and centrifuged for 10 min. The resulting serum was stored at -80° C until analysis. Serum vitamin D, calcium (Ca), alkaline phosphatase (ALP), parathormone (PTH), osteocalcin (OC) and cross-Laps (CL) were measured in the beginning and at the end of the study in both groups.

Serum 25(OH)D was measured using ELISA (Enzyme-linked immunosorbent assay) method (DRG Instruments GmbH, Germany, LOT. No. 070601) on ELISA reader with intraassay CV3.2-5.8%. PTH was measured with Immunotech test kits with intraassay CV 2.8-4.2% and interassay CV 3.2-6.6%.using immunoradiometric assay (IRMA) on a Gamma Counter. Serum concentrations of Ca and ALP were measured by autoanalyser

(HITACHI-902, Germany). An Enzyme-linked immunosorbent assay was used to measure osteocalcin and crossLaps (Nordic Bioscience Diagnostic A/S, Herlev, Denmark). The CV inter and intraassays were 3.6-6.4% and 2.4-3.4% for osteocalcin and 4.1-5.5% and 5.1-5.4% for crossLaps measurement.

Statistical analysis

All statistical procedures were performed using the SPSS package for statistics version 11.5. Values in tables are given as the mean±SD or percentage for quantitative or categorical variables, respectively. To compare the baseline values between groups, we used the student *t*-test. Baseline differences of categorical data were tested by χ^2 analysis. The paired *t*-test was used to compare the values of biochemical parameters at baseline after duration of the study. Two-way *t*-test was used to compare changes of these variables between the groups of study. We used the Pearson bivariate correlation to examine possible correlations between total time of direct sun-exposing and serum biochemical indices in the sun-exposed group after duration of the study. *P*< 0.05 were considered significant.

Results

Subjects aged 27.46±8.78 yr. The skin areas exposed to sunlight was approximately 75% of body surface (14) in the test group. Sunlight intensity was 20-22 Lux during the hours that the test group exposed to it in the study duration.

Two subjects in the control group did not complete the study and were excluded. Five subjects in the test group could not complete the least number of required sessions and withdrew from the study. Totally 125 subjects completed the study (80 subjects in the test group and 45 subjects in the control group).

Demographic and baseline clinical features of the study participants are presented in Table 1. There were no significant differences between the two study groups in age, BMI, usual time spent outdoors in daylight hours, and calcium intake. However, the intake of vitamin D was

higher in the test group ($P= 0.012$). The control group had a higher rate of sunscreen using ($P= 0.017$).

Baseline levels of cross Laps were correlated with the color of skin. Subjects with white skin had a lower level of CL compared to the girls with swarthy and brown skin ($P= 0.006$). There was not a significant association between baseline levels of other serum biochemical indices and time spent outdoors, sunlight exposure and sunscreen usage (Data not shown).

Total time of sun exposing after duration of the study was 16.4 ± 22.0 h in the test group, and degree of skin color changes was "low" in 30%, "medium" in 26%, "high" in 30% and "very high" in 14% of this group.

No significant changes in serum vitamin D and other parameters of bone metabolism were observed in two groups (Table 2). The changes of serum vitamin D, PTH, osteocalcin and cross-Laps concentrations were no different between the groups (Table 3).

In the sun-exposed group, serum vitamin D positively correlated with total time of direct sun exposing after the study ($P= 0.009$). This correlation was very strong in subjects with white skin color ($P= 0.002$) and borderline significant in swarthy-skin subjects ($P= 0.051$). There was no significant correlation between this time and other serum biochemical indices.

Table 1: Baseline characteristics of participants

Characteristics	Group		P
	Sunlight-exposed (n=45)	Control (n= 80)	
Age (years)	27.45±10.26	27.48±4.55	0.175*
BMI (kg/m ²)	21.47±2.19	21.63±3.43	0.858*
Dietary calcium (mg/day)	649.40±309.77	541.20±235.54	0.264*
Dietary vitamin D (IU/day)	456.31±263.01	270.38±132.72	0.012*
Usual time spent outdoors in daylight hours (min/day); %			
<15	35	38.1	0.615†
15-30	36	42.9	
30-60	29	19%	
Sunlight exposure; %			
limited to hand and face	70.6	95	0.012†
more than hand and face	29.4	5	
Usage of sunscreen; %			
Yes	35	76.2	0.017†
Sometimes	45	14.3	
Never	20	9.5	
Color of skin; %			
White	15	49.6	0.068†
Swarthy	55	45.6	
Brown	30	4.8	

Values are presented as mean±SD. Categorical variables are expressed as percentages.

* Unpaired *t*-test.

† χ^2 analysis between sunlight-exposed and control groups.

Table 2: Serum biochemical parameters of bone metabolism levels in two groups before and after the study¹

	Sunlight-exposed group (n=45)		P*	Control group (n= 80)		P*
	Baseline	End of the study		Baseline	End of the study	
Vitamin D (ng/ml)	5.67 ± 10.66	6.06 ± 4.99	0.888	3.46 ± 3.38	5.45 ± 5.45	0.190
Ca (mg/dl)	10.39 ± 0.37	10.24 ± 0.34	0.133	10.13 ± 0.38	10.29 ± 0.42	0.075
ALP (U/L)	145.10 ± 42.42	142.85 ± 33.62	0.711	125.31 ± 54.43	130.08 ± 46.96	0.278
PTH (pg/ml)	26.19 ± 11.42	25.03 ± 10.05	0.665	31.52 ± 10.99	31.31 ± 17.32	0.959
OC (ng/ml)	18.58 ± 6.87	15.51 ± 6.92	0.042	14.78 ± 6.89	14.23 ± 9.33	0.690
CL (ng/ml)	0.72 ± 0.24	0.66 ± 0.22	0.281	0.53 ± 0.24	0.55 ± 0.21	0.662

¹Values are presented as mean±SD. Ca, calcium; ALP, alkaline phosphatase; PTH, parathormone; OC, osteocalcin; CL, crossLaps.

* Paired *t*-test.

Table 3: Changes in serum vitamin D, PTH, OC and CL after duration of the study in two groups¹

	Sunlight-exposed group (n=45)	Control group (n=80)	P*
Vitamin D (ng/ml)	0.39±12.10	1.99±5.38	0.646
PTH (pg/ml)	-1.12±11.85	-0.21±14.64	0.835
OC (ng/ml)	-3.10±5.94	-0.55±4.83	0.217
CL (ng/ml)	-0.06±0.21	0.02±0.15	0.288

¹ Values are presented as mean±SD. PTH, parathormone; OC, osteocalcin; CL, crossLaps.

* Unpaired *t*-test.

Discussion

Vitamin D is recognized as the sunshine vitamin. During exposure to sunlight, UVB radiation is absorbed by 7-dehydrocholesterol that is present in the plasma membranes of both epidermal keratinocytes and dermal fibroblasts, leading to its transformation to previtamin D₃, which is converted to vitamin D₃ (15). After that, vitamin D-binding protein draws it into the circulation.

Despite the well known synthetic processes of vitamin D and its physiology in the body, reasons of the high prevalence of vitamin D deficiency in sunny countries is not clear (7-9, 16). This clinical trial was performed to evaluate direct effects of sunlight on serum vitamin D and bone turnover in healthy young girls.

Anything that either influences the number of solar UVB photons that penetrate the skin influences the cutaneous production of vitamin D₃. It is shown that a person 70 yrs of age exposed to the same amount of sunlight as a 20 yr old person makes ~25% of the vitamin D₃ that the 20 yr old person can make (17). We therefore, performed this study on a group of young girls.

Our study demonstrated that direct sun-exposing had no significant effects on serum vitamin D, bone turnover and other bone metabolism parameters of young girls. Since bone turnover markers are suggested as a valid short-term mar-

ker of bone changes, we measured osteocalcin as a bone formation, and cross Laps as a bone resorption marker. There was a strong correlation between total time of sun-exposing and serum vitamin D levels after the study period in subjects with white skin color in the test group. Serum vitamin D and bone turnover changes were not correlated with other baseline characteristics in sun-exposed group.

Season, time of day, latitude, skin pigmentation, skin coverage, and use of sunscreens are the other factors affecting cutaneous production of vitamin D (4). This study was carried out in Tehran, located in 36° 21' N, and in the warmest months of the year, at the times of days with the highest temperature. Participants were also asked not to use sunscreens during the times of sun-exposure in swimming pool.

Regarding the essential role of sunlight in vitamin D synthesis, it is quite unexpected to see a high prevalence of vitamin D deficiency in countries such as Saudi Arabia (9) and many other sunny countries (7-8, 16). Although there is sufficient sunlight in all seasons in Saudi Arabia, Sedrani showed that half of people who had more than 30 min of sun exposure had vitamin D less than 8 ng/ml (18). We also did not observed any effect of direct sunlight exposure on serum vitamin D in this study.

Tehran has been reported as a city with high prevalence of vitamin D deficiency which is not related to the duration of exposure to sunlight and kind of clothing (10). A high degree of air pollution is suggested as a hypothesis for vitamin D deficiency (10) and lack of sun exposure effect on serum vitamin D in this clinical trial. Even in traditionally sunny countries at low latitudes, the importance of atmospheric pollution on reducing effective UVB levels and vitamin D deficiency has been emphasized (19).

Asian racial of our sample group could be another hypothesis to explain the results of this study. Although sunlight plays an essential role in vitamin D synthesis, its' role in vitamin D deficiency of Asians is not obvious. A higher activity of 24-hydroxylase in fibroblasts of In-

dian-Americans compared to controls has been demonstrated (20). Therefore, increased vitamin D catabolism may cause vitamin D deficiency in Asians. Another study showed similar rate of vitamin D synthesis in Asians as of Europeans; but Asians required greater duration of exposure (21). We observed a strong correlation between total time of sun-exposing and serum vitamin D levels in subjects with white skin color after duration of the study. This correlation was borderline significant in swarthy-skin subjects and not significant in subjects with brown color skin. Harinarayan suggested that hypovitaminosis D in subjects from Tirupati with daily exposure of near-perpendicular tropical sunshine for 4–6 h could be explained by their relatively dark skin complexion (22). Skin pigmentation is one of the reasons for vitamin D deficiency in Delhi, despite abundant sunlight (7).

The association between serum vitamin D and bone markers is reported in some studies. Jones et al. reported that serum 25(OH)D₃ was significantly associated with bone-specific alkaline phosphatase in cut point analysis but not continuous analysis, and was associated with urinary pyridinoline in both forms (23). The study on elderly women with alzheimer's disease in Japan reported that sunlight exposure for 1 yr enhanced 25-OHD levels, which resulted in the correction of hyperparathyroidism and bone turnover. Serum bone Gla protein (BGP) and urinary deoxypyridinoline (D-Pyr) levels, as bone resorption markers, decreased in the exposed group and increased in the deprived group (24). In a study on 72 elderly subjects with vitamin D insufficiency, urinary-free deoxypyridinoline (DPD) was found to correlate with 25(OH)D levels. Supplementation with vitamin D corrected their vitamin D status after 3 months and all markers of bone turnover resorption decreased significantly (25). However, osteocalcin was not found to be related to 25(OH)D levels in elderly people (25, 26) and in pre-menopausal women (27). Since the levels of serum vitamin D was not affected by sunlight exposure, lack of any alteration in bone markers concentrations could be speculated.

There were some limitations to the present study. Dietary vitamin D intake and subjects' distribution according to their baseline skin color were different in the groups of the study. Although these differences between the study groups could not affect our main results relating to the vitamin D and bone markers, however, it should be considered in comprehensive studies in this field. Limitation in UVB radiation measurement was another restriction in this study.

In summary, our results show that sun exposure did not influence enough serum vitamin D and bone turnover parameters in healthy young girls in Tehran. This could be partially attributed to the heavy atmospheric pollution or our subjects' Asian racial with a probably lower capacity of vitamin D synthesis from sunlight. This should emphasize the need for vitamin D fortification of foods in order to obtain adequate vitamin D (28–30). However, subjects with bright skin complexion benefit more from sunlight exposing compared to those with a dark skin color in the case of vitamin D improvement.

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