Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2016) 46: 72-78

doi:10.3906/sag-1403-114

Seasonal vitamin D status and endothelial function in healthcare workers

Özlem HALİLOĞLU[1,*], Tolga KASACI[2], Dilek YAVUZ[3]

[1]Department of Internal Medicine, Faculty of Medicine, Marmara University, İstanbul, Turkey
[2]Department of Biochemistry, Faculty of Medicine, Marmara University, İstanbul, Turkey
[3]Department of Endocrinology and Metabolism, Faculty of Medicine, Marmara University, İstanbul, Turkey

Received: 21.03.2014 • **Accepted/Published Online:** 18.06.2015 • **Final Version:** 05.01.2016

Background/aim: Healthcare workers have long working hours indoors and are at risk of vitamin D deficiency. The aim of this study was to determine seasonal vitamin D status and its relationship with early atherosclerotic markers, endothelial function, and carotid intima-media thickness (CIMT) in healthcare workers of Marmara University Hospital, İstanbul.

Materials and methods: One hundred and ninety healthy volunteer healthcare workers and 66 nonmedical volunteers of Marmara University Hospital were included in the study and 25-hydroxyvitamin D (25(OH)D), calcium, intact parathyroid hormone (PTH), endothelial function, and CIMT were measured twice during winter and summer seasons.

Results: Mean vitamin D levels were 20 ng/mL in summer and 16.4 ng/mL in winter. Out of the healthcare workers, 48.9% were vitamin D deficient at the end of summer and 71.5% in winter. Flow-mediated dilation (FMD) values were similar in both groups in both seasons; however, FMD values of 64 healthcare workers in summer were significantly higher than in winter. Serum 25(OH)D was positively associated with FMD ($r = 0.1797$, $P = 0.0441$) and negatively correlated with serum PTH ($r = -0.2459$, $P < 0.0001$). A negative correlation between FMD and serum PTH ($r = -0.1757$, $P = 0.0473$) was observed.

Conclusion: Vitamin D levels of healthcare workers are very low, even in summer time. Healthcare workers must be considered a group at major risk for vitamin D deficiency.

Key words: Vitamin D deficiency, healthcare worker, 25-hydroxyvitamin D, endothelial function, carotid intima media thickness

1. Introduction

Vitamin D deficiency is common all over the world and the frequency is gradually increasing. Vitamin D deficiency is seen not only in countries with low sun exposure but also in sunny countries (1–3). Recent studies indicated that decreased sun exposure (because of decreased outdoor activity, immobility, and long working hours indoors), increased sunscreen use and sun avoidance, low dietary intake, and increased obesity are major reasons behind the increasing prevalence of vitamin D deficiency (4,5).

Besides its very well-known musculoskeletal effects, vitamin D has some other effects on the extraskeletal system. There are studies showing that vitamin D prevents some cancer types and decreases mortality, has some immunomodulatory effects on both innate and acquired immune systems and decreases autoimmune diseases, increases insulin release via decreasing pancreatic β-cell destruction, and decreases insulin resistance (6–13).

Vitamin D also has beneficial effects on cardiovascular health (13). Endothelial function and carotid-intima media thickness (CIMT) are two parameters that are accepted as early markers of atherosclerosis. There are studies demonstrating that vitamin D deficiency is associated with endothelial dysfunction and CIMT (14,15).

Healthcare workers work long hours indoors and cannot take enough sunlight; they are an important risk group for vitamin D deficiency. A study from the United States showed that 74% of internal medicine residents have vitamin D levels below 50 nmol/L at the end of winter (16). In a study from a sunny country, Qatar, 97% of 340 healthcare workers had vitamin D levels below 30 ng/mL (17). Another study from India showed that 66% of healthcare workers have low vitamin D levels (18). According to our knowledge, there are no studies showing the vitamin D levels of healthcare workers in Turkey.

Turkey is located at 36°N–42°N and 26°E–45°E and İstanbul is located at the 41°N. Due to İstanbul's location and geographic features, it can be considered a "sunny" city: from 1100–1500 hours between May and October, there is adequate sunlight for the production of vitamin D.

* Correspondence: ozlemasmaz@gmail.com

This study aimed to determine the vitamin D status and seasonal changes in healthcare workers at Marmara University Hospital in İstanbul and to study the relationships of vitamin D with endothelial function and CIMT, which are early risk factors of cardiovascular health.

2. Materials and methods

2.1. Subject selection

One hundred and ninety volunteer healthcare workers, including medical doctors, nurses, and assistant medical staff, working at Marmara University Hospital, aged between 21 and 57 years, were included in the study. Healthcare workers with systemic inflammatory diseases or malignancy, who were taking medication that interfered with vitamin D metabolism, or who had hepatic or renal dysfunction were excluded from the study. Sixty-six age- and sex-matched nonmedical volunteers were included as the control group.

The study was approved by the local ethics committee of the Marmara University School of Medicine. It was funded by a grant from the Marmara University Research Foundation (Grant No.: SAG – C – TUP – 090909 – 0282). Informed consent was obtained from all subjects.

2.2. Study protocol

This prospective observational study was conducted at Marmara University Hospital in İstanbul, Turkey. All the questionnaires and measurements were taken twice. Winter data were taken in March and April 2010 and summer data in September and October 2010.

Height, weight, body mass index (BMI), and systolic and diastolic blood pressures were measured. Questionnaires about daily physical activity, sunlight exposure (sunlight exposure was quantified as UV index), and daily calcium intake were administered. Venous blood samples were collected early in the morning after 8 h of fasting. Serum samples were stored at –80 °C until studied. Sixty-four of the 190 subjects were evaluated for endothelial function and CIMT measurements (19).

2.3. Biochemical parameters

The 25-hydroxyvitamin D (25(OH)D) concentrations were measured by high-performance liquid chromatography method (Recipe Chemicals, Germany). Intraassay and interassay coefficients of variation were 0.7%–4.9% and 3.1%–4.7%, respectively. Vitamin D deficiency was considered as 25(OH)D of <20 ng/mL, insufficiency as 25(OH)D of 20–29 ng/mL, and sufficiency as 25(OH)D of ≥30 ng/mL (20). Serum intact parathyroid hormone (PTH) was determined by use of immunochemiluminescence assay (Modular Analytics E170; Roche Diagnostics, Germany). Intraassay and interassay coefficients of variation were 1.1%–2% and 2.5%–3.4%, respectively. Calcium and phosphorus concentrations were measured with an enzymatic colorimetric assay method (Roche Diagnostics). Intraassay and interassay coefficients of variation were 0.4%–0.9% and 0.7%–1.5% for the calcium kit and 0.7%–0.9% and 1.4%–1.7% for the phosphorus kit, respectively.

2.4. Endothelial function assessment

Endothelial function was evaluated by high-resolution Doppler ultrasonography with a 12 MHz linear transducer (LOGIQ3 Expert; GE, USA) from the right brachial artery, measuring flow-mediated dilation (FMD) as described before.

All measurements were performed under the observation of a cardiologist by the same author, whose intraobserver variation was less than 2%. Measurements were taken in the morning at 20–25 °C room temperature after 8 h of fasting. The brachial artery was evaluated at the longitudinal view 2 cm above the elbow. Before the evaluation, the blood pressure of the subject was assessed. After measuring the basal diameter of the brachial artery, a sphygmomanometer cuff was placed on the forearm. Arterial occlusion was created by inflating the cuff to 50 mmHg above the subject's systolic blood pressure for 5 min. Lumen diameter was noted 1 min after the cuff was deflated to assess FMD. FMD was expressed as the percentage change in vessel diameter during reactive hyperemia. Intraobserver variation in FMD measurement was 10% (21).

2.5. CIMT assessment

CIMT was evaluated by high-resolution Doppler ultrasonography with a 12 MHz LOGIQ3 linear transducer (LOGIQ3 Expert).

While the subject was lying down, an image where the common carotid artery, carotid bifurcation, and internal and external carotid arteries were all visible was viewed first. The double line pattern of the intima-media layer 1 cm proximal to carotid bifurcation was determined and 5–8 measurements were taken from the vessel wall far from the ultrasound guidance probe. The means of the 3 highest values were recorded and used for statistics (19).

2.6. Questionnaires

2.6.1. Daily physical activity

Daily physical activity score was graded according to the participants' waking hours, vehicle that was used for coming to work, the physical strength required for their work, and time spent while sitting. All points of the questions were added and the total score was graded as "highly active lifestyle": 16–20 points, "medium physical activity": 12–15 points, and "sedentary lifestyle": <11 points.

2.6.2. UV index

The clothing styles ('wearing' score) and the average time spent in the sun per day ('sunlight exposure' score) of the participants were determined and scored. The 'wearing' score was calculated according to the percentage of body parts covered by the participants' usual clothes. The 'sunlight exposure' score was graded as: "No direct sunlight exposure": 1 point, "sunlight exposure except 1100–1500 hours": 2 points, "sunlight exposure between 1100 and 1500 hours irregularly": 3 points, "sunlight exposure everyday regularly at least 30 min between 1100 and 1500 hours": 4 points, and "sunlight exposure all day": 5 points. UV indices were calculated by dividing the 'sunlight exposure' score by the 'wearing' score.

2.6.3. Daily calcium intake

All the participants filled out a form regarding their consumption of calcium-rich foods, such as milk, yogurt, cream, cheese, breads and grains, fish, desserts, nuts, vegetables, and fruits. The total daily consumption of calcium was calculated by the International Osteoporosis Foundation's calcium calculator program.

2.7. Statistical analysis

SPSS 15.0 for Windows (SPSS Inc., USA) was used for the analyses. Comparisons between the groups were made with the paired t-test and ANOVA where appropriate. Pearson or Spearman correlations with two-tailed probability values were used for parametric and nonparametric values, respectively; two-tailed probability values were used to estimate the strength of association between variables. The Pearson correlation with two-tailed probability values was used to estimate the strength of associations between variables. A stepwise multiple regression analysis was performed to define the predictors of CIMT. The level of statistical significance was set at $P < 0.05$. All results were expressed as mean ± SD.

3. Results

The demographic characteristics of the subjects and controls were not different in terms of age (34.7 ± 7.68 versus 32.47 ± 8.21) and sex (M/F: 98/92 versus 35/31).

There were no statistically significant seasonal differences in BMI, systolic and diastolic blood pressure, physical activity, UV indices, or daily calcium intakes.

Among 92 women healthcare workers, there was no woman veiled, and among the controls there was only one. Her 25(OH)D levels were below 10 ng/mL in both seasons.

Biochemical parameters are shown in the Table. PTH levels were higher at the end of winter than summer in both healthcare workers and the nonmedical volunteers. Plasma 25(OH)D levels at the end of winter were similar between groups, whereas they were higher in the control group than in the study group at the end of summer. When the seasonal change was evaluated, 25(OH)D levels were significantly lower in both groups in winter than in summer.

In the healthcare worker group, 12.6% of the subjects had normal serum 25(OH)D levels (>30 ng/mL) at the end of summer and only 3.9% at the end of winter; this difference was statistically significant, as shown in Figure 1 ($P = 0.0003$). In the nonmedical volunteer group, normal 25(OH)D levels were 57.7% and 33.3% in summer and winter, respectively, and this difference was also statistically significant ($P < 0.01$). When the healthcare workers and the nonmedical volunteers were compared according to the seasons, both the summer and the winter 25(OH)D levels of healthcare workers were lower than the nonmedical volunteer group ($P < 0.01$).

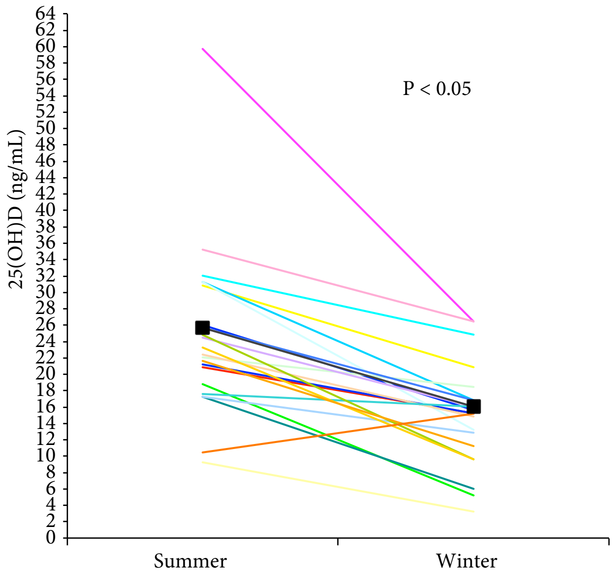

Figure 1. Individual changes in 25(OH)D levels of healthcare workers from summer to winter.

Table. Biochemical data of the study groups.

	Healthcare workers (n=190)		P	Control group (n=66)		P
	Summer	Winter		Summer	Winter	
25(OH)D (ng/mL)	20.25 ±9.12*	16.80 ±8.35	<0.05	36.2 ±12.6	21.1 ±13.3	0.001
PTH (pg/mL)	25.49 ±9.61	32.97 ±10.98	<0.001	29.2 ±10	40.5 ±12.0	<0.01
Ca (mg/dL)	8.90 ±0.41	8.93 ±0.45	n.s.	9.0 ±0.7	8.9 ±0.6	n.s.
P (mg/dL)	3.48 ±0.47	3.50 ±0.53	n.s.	3.6 ±0.4	3.6 ±0.5	n.s.

When the healthcare workers were classified according to their occupation, the serum 25(OH)D levels of medical doctors were significantly higher at the end of summer than winter (23.22 ± 9.73 versus 17.13 ± 8.99; $P < 0.0001$); but there was no seasonal difference found in the nurse or assistant medical staff subgroups. The summer 25(OH)D levels of medical doctors were significantly higher than those of the nurses (23.22 ± 9.73 versus 16.72 ± 6.71; $P < 0.001$) and assistant medical staff (23.22 ± 9.73 versus 16.46 ± 7.22; $P < 0.01$) subgroups, respectively, but there was no difference found in winter levels. When the healthcare workers were classified according to their working environments, there was no difference in vitamin D levels between subjects working in the medical and surgical sciences ($P > 0.05$).

The FMD levels were significantly higher at the end of summer than winter in both groups (11.53 ± 6.07% and 8.79 ± 4.75%, $P = 0.008$ in healthcare workers versus 11.2 ± 5.1% and 7.0 ± 3.3%, $P = 0.001$ in nonmedical volunteers). Subgroup analysis of the healthcare workers indicated that there was a significant seasonal difference in FMD levels of medical doctors (11.65 ± 6.28% at the end of summer versus 8.83 ± 4.95% at the end of winter, $P = 0.01$), but no difference was found in other subgroups ($P > 0.05$).

When CIMT levels were evaluated, there were no statistically significant differences found between groups or seasons, whereas there was a significant seasonal change observed in the medical doctor subgroup (0.42 ± 0.06 mm at the end of summer versus 0.44 ± 0.06 mm at the end of winter, $P = 0.01$).

Statistically significant correlation analyses are shown in Figure 2. No significant correlation was found between serum 25(OH)D levels and CIMT ($r = -0.15$, $P = 0.08$).

Linear regression analysis was performed to determine the predictors of CIMT. BMI, age, sex, daily calcium intake, 25(OH)D, PTH, and FMD were included in the linear regression model. PTH, 25(OH)D, and FMD were shown to significantly and independently affect CIMT ($R^2 = 10.03\%$, $P = 0.007$) in this study.

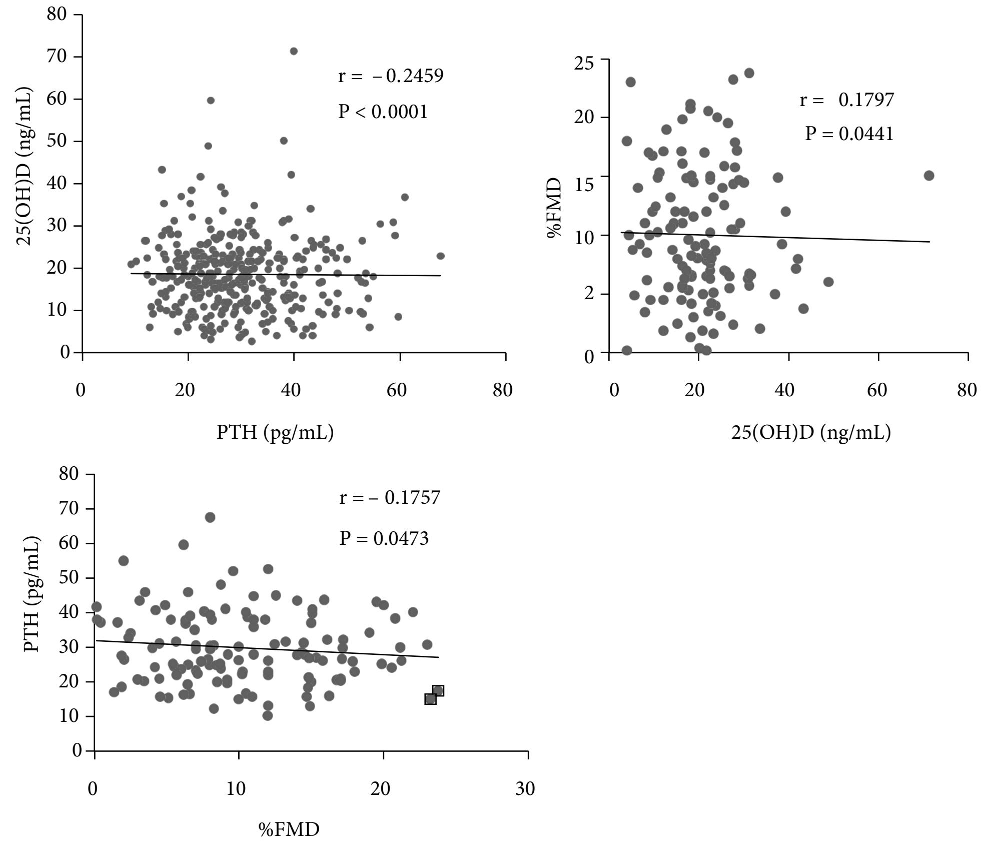

Figure 2. The correlation data of the healthcare workers showed a negative correlation between 25(OH)D and PTH levels ($r = -0.2459$; $P < 0.0001$), also between FMD and PTH values ($r = -0.1757$; $P = 0.0473$). There was a positive correlation between 25(OH)D levels and FMD values ($r = 0.1797$; $P = 0.0441$).

4. Discussion

In this study, we demonstrated that vitamin D levels of healthcare workers at a university hospital in İstanbul were lower than the predicted levels in both summer and winter. To our knowledge, this is the first study evaluating the vitamin D levels of healthcare workers in Turkey.

There are few studies evaluating the vitamin D levels of healthcare workers in the literature. A study from Boston Medical Center reported that 32% of healthy students, physicians, and residents were found to be vitamin D deficient (22). Another study from India found that 66% of healthcare workers have low vitamin D levels (18). A study from Qatar revealed that 96.5% of healthcare workers, including medical doctors and nurses, have 25(OH)D levels below 30 ng/mL (17).

In our study, we determined that the healthcare workers were indoors from 1100 to 1500 hours, which is when UV rays are the most effective.

Vitamin D supplementation of foods is one hypovitaminosis D prevention strategy in some countries, such as Canada and the United States. In Turkey, however, vitamin D supplementation in foods is still controversial. We also showed in our study that the number of subjects taking dietary vitamin D supplements was very low; this could be a reason why the hypovitaminosis D rate in this study was so high.

Besides the sun exposure and vitamin D supplementation data, we found that not only the healthcare workers but also the control group had very low daily calcium intakes compared to that recommended by the Institute of Medicine committee (23). We determined that both the healthcare workers and the control group of our study needed calcium supplementation to reach the recommended dose.

Furthermore, the vitamin D levels of healthcare workers were lower at the end of winter than at the end of summer. This is consistent with the literature (24–26).

A very recent study from Turkey comparing the seasonal variation of the 25(OH)D levels in office workers showed that the vitamin D levels of office workers in winter were lower than in the summer and that vitamin D deficiency is very prevalent, even in summertime, in office workers in Ankara (27). In our study, we also observed seasonal variation of the 25(OH)D levels. Consistent with the study from Ankara, we also found that the vitamin D levels were normal in only 12.6% of cases, even in summertime, in İstanbul.

It is already known that vitamin D has effects on the cardiovascular system. One of these effects is on blood pressure. Some studies showed that there is an inverse relationship between 25(OH)D levels and blood pressure (13,28,29), but besides these studies, there is a metaanalysis consisting of 10 studies showing that there is no statistically significant correlation between these two (30). In our study, we could not find a relationship between serum 25(OH)D levels and systolic and diastolic blood pressures, respectively.

In the literature, there are some studies showing the relationship between 25(OH)D levels and endothelial function. Al Mheid et al. reported that vitamin D levels were associated with FMD in healthy adults (31). Another study showed that low 25(OH)D levels were associated with endothelial dysfunction in middle-aged and older adults (32). Additionally, in a study from Turkey, asymptomatic vitamin D deficient subjects were evaluated before and after treatment for FMD; after treatment with vitamin D, they reported an improvement in endothelial function (33). Similar to these studies, we also determined a positive correlation between serum 25(OH)D and FMD in our study. We also found a relationship between seasonal variation of vitamin D levels and endothelial function in both the healthcare worker and the nonmedical volunteer groups.

When healthcare workers were analyzed according to their occupation, we found that medical doctors had a statistically significant seasonal variation of FMD and CIMT levels. FMD levels were higher and CIMT levels were lower in summer than in winter. This variation seems to be related to the more depressed 25(OH)D and increased PTH levels of the medical doctors' subgroup; all these findings could be due to the longer indoor hours and the more frequent and long shifts of the medical doctors when compared with the nurses and the assistant medical staff.

Although there are some studies showing an inverse relationship between serum 25(OH)D levels and CIMT (34,35), some very recent studies showed that vitamin D status and supplementation have no relationship with CIMT (36). In our study, we also could not find a statistically significant association between them in either group.

One of the limitations of our study is the small number of participants and controls. Another one is that although FMD and CIMT are clearly identified parameters of cardiovascular risk, it could be better to support these findings with 10-year clinical cardiovascular risk calculations and also with other well-known risk markers, such as hsCRP, IL-10, plasminogen activation inhibitor-1, and circulating endothelial progenitor cell counting, in order to make the cardiovascular risk evaluation more sensitive.

In conclusion, the vitamin D levels of healthcare workers at Marmara University Hospital in İstanbul, Turkey, is low at the end of summer and winter. Although vitamin D levels were higher in summer than in winter, even the summer levels were approaching vitamin D deficiency. Only 4% of the healthcare workers had normal levels of vitamin D, so this group should be considered at risk for vitamin D deficiency. Low vitamin D levels have negative effects on cardiovascular health and the relationship between vitamin D levels and FMD, which is an early marker of atherosclerosis, makes this clear. Large and multicenter studies are needed and healthcare workers should be routinely screened for vitamin D levels and be given vitamin D supplements as suggested by the guidelines on endocrinology and metabolism on vitamin D.

Acknowledgment

The study was made possible by a grant from the Marmara University Research Foundation.

References

1. Goswami R, Gupta N, Goswami D, Marwaha RK, Tandon N, Kochupillai N. Prevalence and significance of low 25-hydroxyvitamin D concentrations in healthy subjects in Delhi. Am J Clin Nutr 2000; 72: 472–475.
2. McGrath JJ, Kimlin MG, Saha S, Eyles DW, Parisi AV. Vitamin D insufficiency in south-east Queensland. Med J Aust 2001; 174: 150–151.
3. Gannage-Yared MH, Chemali R, Yaacoub N, Halaby G. Hypovitaminosis D in a sunny country: relation to lifestyle and bone markers. J Bone Miner Res 2000; 15: 1856–1862.
4. Binkley N, Ramamurthy R, Krueger D. Low vitamin D status: definition, prevalence, consequences, and correction. Endocrinol Metab Clin North Am 2010; 39: 287–301.
5. Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988–2004. Arch Intern Med 2009; 169: 626–632.
6. Holick MF. Vitamin D: extraskeletal health. Endocrinol Metab Clin North Am 2010; 39: 381–400.
7. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: results of a randomized trial. Am J Clin Nutr 2007; 85: 1586–1591.
8. Garland CF, Garland FC, Gorham ED. Can colon cancer incidence and death rates be reduced with calcium and vitamin D? Am J Clin Nutr 1991; 54: 193S–201S.
9. Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, Hankinson SE. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2005; 14: 1991–1997.
10. Bikle D. Nonclassic actions of vitamin D. J Clin Endocrinol Metab 2009; 94: 26–34.
11. Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. Am J Clin Nutr 2004; 80: 1717S–1720S.
12. Tanaka Y, Seino Y, Ishida M, Yamaoka K, Satomura K, Yabuuchi H, Imura H. Effect of 1,25-dihydroxyvitamin D3 on insulin secretion: direct or mediated? Endocrinology 1986; 118: 1971–1976.
13. Bouillon R. Vitamin D as potential baseline therapy for blood pressure control. Am J Hypertens 2009; 22: 816.
14. Lane HA, Smith JC, Davies JS. Noninvasive assessment of preclinical atherosclerosis. Vasc Health Risk Manag 2006; 2: 19–30.
15. O'Leary DH, Polak JF. Intima-media thickness: a tool for atherosclerosis imaging and event prediction. Am J Cardiol 2002; 90: 18L–21L.
16. Haney EM, Stadler D, Bliziotes MM. Vitamin D insufficiency in internal medicine residents. Calcif Tissue Int 2005; 76: 11–16.
17. Mahdy S, Al-Emadi SA, Khanjar IA, Hammoudeh MM, Sarakbi HA, Siam AM, Abdelrahman MO. Vitamin D status in health care professionals in Qatar. Saudi Med J 2010; 31: 74–77.
18. Arya V, Bhambri R, Godbole MM, Mithal A. Vitamin D status and its relationship with bone mineral density in healthy Asian Indians. Osteoporos Int 2004; 15: 56–61.
19. Touboul PJ, Hennerici MG, Meairs S, Adams H, Amarenco P, Bornstein N, Csiba L, Desvarieux M, Ebrahim S, Fatar M et al. Mannheim carotid intima-media thickness consensus (2004–2006). An update on behalf of the Advisory Board of the 3rd and 4th Watching the Risk Symposium, 13th and 15th European Stroke Conferences, Mannheim, Germany, 2004, and Brussels, Belgium, 2006. Cerebrovasc Dis 2007; 23: 75–80.
20. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911–1930.
21. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002; 39: 257–265.
22. Tangpricha V, Pearce EN, Chen TC, Holick MF. Vitamin D insufficiency among free-living healthy young adults. Am J Med 2002; 112: 659–662.
23. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011; 96: 53–58.

24. Stamp TC, Round JM. Seasonal changes in human plasma levels of 25-hydroxyvitamin D. Nature 1974; 247: 563–565.

25. Vanderschueren D, Gevers G, Raymaekers G, Devos P, Dequeker J. Sex- and age-related changes in bone and serum osteocalcin. Calcif Tissue Int 1990; 46: 179–182.

26. 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: 1232–1236.

27. Cinar N, Harmanci A, Yildiz BO, Bayraktar M. Vitamin D status and seasonal changes in plasma concentrations of 25-hydroxyvitamin D in office workers in Ankara, Turkey. Eur J Intern Med 2013.

28. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension 2007; 49: 1063–1069.

29. Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. Am J Hypertens 2007; 20: 713–719.

30. Witham MD, Nadir MA, Struthers AD. Effect of vitamin D on blood pressure: a systematic review and meta-analysis. J Hypertens 2009; 27: 1948–1954.

31. Al Mheid I, Patel R, Murrow J, Morris A, Rahman A, Fike L, Kavtaradze N, Uphoff I, Hooper C, Tangpricha V et al. Vitamin D status is associated with arterial stiffness and vascular dysfunction in healthy humans. J Am Coll Cardiol 2011; 58: 186–192.

32. Jablonski KL, Chonchol M, Pierce GL, Walker AE, Seals DR. 25-Hydroxyvitamin D deficiency is associated with inflammation-linked vascular endothelial dysfunction in middle-aged and older adults. Hypertension 2011; 57: 63–69.

33. Tarcin O, Yavuz DG, Ozben B, Telli A, Ogunc AV, Yuksel M, Toprak A, Yazici D, Sancak S, Deyneli O et al. Effect of vitamin D deficiency and replacement on endothelial function in asymptomatic subjects. J Clin Endocrinol Metab 2009; 94: 4023–4030.

34. Targher G, Bertolini L, Padovani R, Zenari L, Scala L, Cigolini M, Arcaro G. Serum 25-hydroxyvitamin D3 concentrations and carotid artery intima-media thickness among type 2 diabetic patients. Clin Endocrinol (Oxf) 2006; 65: 593–597.

35. Carrelli AL, Walker MD, Lowe H, McMahon DJ, Rundek T, Sacco RL, Silverberg SJ. Vitamin D deficiency is associated with subclinical carotid atherosclerosis: The Northern Manhattan study. Stroke 2011; 42: 2240–2205.

36. Blondon M, Sachs M, Hoofnagle AN, Ix JH, Michos ED, Korcarz C, Gepner AD, Siscovick DS, Kaufman JD, Stein JH et al. 25-Hydroxyvitamin D and parathyroid hormone are not associated with carotid intima-media thickness or plaque in the multi-ethnic study of atherosclerosis. Arterioscler Thromb Vasc Biol 2013; 33: 2639–2645.