Clinical implications of vitamin D deficiency

Beata Matyjaszek-Matuszek[1], Monika Lenart-Lipińska[1,2], Ewa Woźniakowska[3]

[1]Department of Endocrinology, Medical University of Lublin, Lublin, Poland
[2]Department of Laboratory Diagnostics, Medical University of Lublin, Lublin, Poland
[3]3rd Chair and Department of Gynecology, Medical University of Lublin, Lublin, Poland

Abstract

Vitamin D deficiency is a common medical problem worldwide and its prevalence rises along with latitude, obesity, sedentary lifestyle, limited sunlight exposure and aging. A great body of evidence has shown that patients with vitamin D deficiency have increased cardiovascular risks and total mortality. Conversely, the presence of comorbidities progressive with age such as abdominal obesity, insulin resistance, type 2 diabetes and hypertension places the patients at an increased risk of vitamin D deficiency. The multidirectional effect of vitamin D deficiency is present in different phases of the aging process. Based on the literature review, the risk factors for vitamin D insufficiency most often found in post-menopausal women include limited sun exposure and time spent outdoors, inadequate dietary vitamin D intake, winter season and increased age. Vitamin D supplementation in this group might offer prevention of falls and fractures and may be beneficial for cardiovascular health, what may be especially important in osteoporotic and elderly populations. Prevention and treatment processes involve education regarding sunlight exposure and pharmacological cholecalciferol supplementation according to the recommendations for Central Europe. This manuscript reviews the role of vitamin D and its deficiency and considers their clinical implications, with particular regard to peri- and postmenopausal women.

Key words: vitamin D, deficiency, perimenopause, menopause.

Introduction

In recent years, vitamin D (VD), a group of steroid compounds, has become of great interest due to many studies which have revealed its role far beyond bone metabolism. Through decades VD was considered a vitamin but nowadays it has emerged as an active hormone exerting its action as a transcription factor regulating the expression of numerous genes [1]. The presence of specific VD receptors (VDR) outside the skeletal system, enterocytes and renal tubular cells was confirmed in many cell types including immune cells, neurons, pancreatic cells, myocytes, cardiomyocytes, endothelium cells, which stress pleiotropic activity of VD. There is a great body of evidence confirming that apart from its well-known function in calcium-phosphate homeostasis, VD also exerts many non-calcemic actions in various tissues and systems. Vitamin D deficiency has been linked with significant complications such as cardiovascular events, obesity, metabolic syndrome, type 2 diabetes, various types of cancer, immune disorders, increased mortality and adverse pregnancy outcomes [2-4].

Currently, VD deficiency is considered a public health problem worldwide and its prevalence rises along with latitude, aging, sedentary lifestyle and limited sunlight exposure due to staying indoors or using sunscreen products. Furthermore, an increasing epidemic of obesity, which results in sequestration of VD in adipose tissue, also contributes to an increased risk of VD deficiency [2, 5].

This manuscript reviews clinical implications of VD deficiency, its prevention and treatment, with particular regard to peri- and postmenopausal women.

Vitamin D metabolism

There are three ways to maintain adequate VD status: sunshine exposure, dietary intake and pharmaceutical supplementation. The major source of VD is its skin synthesis under the influence of solar ultraviolet B (UV-B) radiation which accounts for 80-90% of VD in humans [1]. In comparison to skin synthesis, the dietary supply of VD is minor and amounts only to 10-20% of total VD, however, it can become a significant source of VD if enriched with supplementation [1]. There are two types of physiologically important vitamins D: cholecalciferol (VD_3) mainly synthesized in the skin from 7-dehydrocholesterol upon exposure to UV-B and ergocalciferol (VD_2) obtained from diet. In the liver, VD is metabolized to 25-hydroxyvitamin D-25(OH)D by

VD 25-hydroxylase (25-OHase) and further circulates to the kidneys where it undergoes a second hydroxylation by 25-hydroxyvitamin D-1αhydroxylase (1αOHase) to the biologically active form of VD – 1,25(OH)$_2$D (calcitriol). The hydroxylation of VD in a position 1α is the key regulatory step in the bioactivation of VD and enzyme action in the kidneys is tightly controlled by numerous factors including serum parathormone (PTH), calcium, phosphate and various hormones such as estrogens, androgens, growth hormone, prolactin, thyroxin, cortisol and insulin [1]. The best indicator of the overall VD status is serum 25(OH)D concentration due to its long half-life (1-3 months) and metabolism which reflects total VD from dietary intake and sunlight exposure [6]. Although 1,25(OH)$_2$D is more potent than 25(OH)D and has greater affinity to VDR it should never be used to determine VD status. This compound is present in circulation in a much lower concentration than 25(OH)D and has very short half-life (< 4 hours). Furthermore, the 1,25(OH)$_2$D level may remain within the reference range or may even be elevated in the course of VD deficiency or secondary hyperparathyroidism [7]. The active form of VD combines with VDR and it has been established that VDRs are expressed in numerous tissues and cells. Accumulating evidence has shown that approximately 3% of the human genome may be regulated by VD, which indicates that VD deficiency is associated with significant clinical consequences [8].

The main causes of VD deficiency are limited skin synthesis due to inadequate sunlight exposure caused by sunscreen products overuse or limited outdoor activity as well as a low dietary intake of VD rich foods and many other factors such as aging, pigmented skin, smoking, obesity, air pollution, malabsorption and reduced synthesis due to liver or kidney diseases [7].

Diagnostic criteria of vitamin D deficiency

Serum 25(OH)D concentration is the best parameter to assess the overall VD status [9]. Current International Osteoporosis Foundation Guidelines recommended a target level of 30 ng/ml, which is associated with maximal suppression of PTH [9]. The diagnostic criteria of VD status are presented in Table I. There are several commercial assays available for the assessment of 25(OH)D which produce reliable results, but have notable bias in comparison with the reference HPLC method. It is worth knowing that the variation between laboratories may be as high as 30%. Liquid chromatography tandem mass spectroscopy (LC-MS) is considered the gold standard for the evaluation of VD status, due to commercially available methods such as RIA, ELISA, chemiluminescence assay may not measure all circulating forms of VD [11, 12].

Vitamin D and obesity

The link between obesity and VD deficiency has been observed for years but determining the cause and effect has been difficult. Vimaleswaran et al. suggested that a higher BMI leads to a lower VD status whereas the effects of low VD status on BMI are likely to be marginal. In other words, these findings provide evidence for obesity as the causal factor for the development of VD deficiency but there is no proof that VD deficiency serves as the causal factor for the development of obesity [13]. Nonetheless, experimental studies have demonstrated that 1,25(OH)$_2$D$_3$ plays an active role in adipose tissue by modulating inflammation, adipogenesis and adipocyte secretion as the key component of metabolic disorders e.g. in the metabolic syndrome [14]. A large study of the genetics underpinning both conditions finds that obesity may decrease VD levels but a predisposition to VD deficiency does not in fact lead to obesity. The findings also suggest that increasing VD levels will not reverse obesity. The fundamental mechanism that would explain why obesity suppresses VD is still discussed. Since VD is fat soluble, some scientists had assumed that it was sequestered in fatty tissues. If this was the case, less VD would reach the bloodstream. Nevertheless, while the vitamin is indeed stored in the adipose tissue, there is no evidence for sequestration of supplemental or endogenous cholecalciferol. Dilution of ingested or cutaneously synthesized VD in the large adipose tissue of obese patients fully explains their typically low VD status [15]. Therefore, the patients with BMI over 30 may require higher or more frequent doses of VD [16]. Nonetheless, Mason et al. found that vitamin D$_3$ supplementation during weight loss did not translate into higher body mass reduction or associated factors as compared with placebo, however, women who became replete experienced greater improvements [17].

Vitamin D and diabetes risk

Previous studies have yielded contradictory findings on the relationship between low VD and impaired

Tab. I. Diagnostic criteria of vitamin D status [10]

	Severe deficiency	Deficiency	Insufficiency	Optimal concentration	Risk of toxicity
25(OH)D$_3$ concentration (ng/ml)	0-10	10-20	20-30	30-80	> 100

glucose homeostasis. However, calcium is necessary to secrete insulin, which indirectly suggests that VD may in fact contribute to maintaining insulin secretion. Among the disorders linking VD deficiency to hyperglycemia we can find type 1 (T1DM), type 2 diabetes mellitus (T2DM) and metabolic syndrome. Type 1 diabetes mellitus and T2DM patients have a higher incidence of VD deficiency in comparison to the healthy population. The insulin-producing β-cells as well as numerous cell types of the immune system express VDR and vitamin D-binding protein and some allelic variations in genes involved in VD metabolism. What is more, its receptors are linked with glucose intolerance, insulin secretion and sensitivity as well as inflammation [18]. In the case of T1DM, VD supplementation in pre-diabetic individuals could help prevent or at least reduce the onset of autoimmune processes possibly by regulating thymic selection of the T-cell repertoire, decreasing the numbers of auto-reactive T cells and inducing Treg cells. Although immune modulation is widely discussed in the treatment of T1DM, it is also relevant in T2DM [19]. What is more, pharmacologic doses of 1,25-dihydroxyvitamin D $(1,25(OH)_{(2)}D)$ prevent insulitis and T1DM in non-obese diabetic mice and other models of T1DM. The possible reason behind this process may be immune modulation as well as direct effects on β-cell function [19, 20]. Normal VD levels in the mother, and consequently in the fetus, decrease the risk of T1DM development in the child. The risk rises when maternal VD deficiency occurs in the second trimester as at this time i.e. around the 12th week pancreatic β-cells are formed; while insulin secretion begins in the 20th week of gestation. For that reason the mother should begin VD supplementation in the second trimester of gestation at the latest [21]. So far VD insufficiency has been known to serve as a T1DM risk factor, however, a growing body of evidence has pointed to its role in T2DM development. Vitamin D ameliorates the harmful biochemical impact of T2DM, possibly by increasing insulin secretion and sensitivity, improving the β-cell function, and decreasing the number of pro-inflammatory cytokines and insulin resistance [22]. Teegarden et al. confirmed that VD – or its active metabolite 1,25-dihydroxyvitamin D $(1,25(OH)_2D)$ – improves insulin sensitivity even in patients with glucose metabolism parameters with normal ranges. The proposed mechanisms possibly underlying this effect include potential relationships with improvements in lean mass, regulation of insulin release, altered insulin receptor expression and specific effects on insulin action [23]. Kramer et al. recognized VD deficiency and insufficiency with increased PTH is an independent predictor of such disorders as β-cell dysfunction, insulin resistance, and glycemia. The authors highlighted the need to consider the PTH/25-OH-D axis when studying the impact of VD status on glucose homeostasis [24]. Cho et al. showed VD 25(OH)D

< 20 ng/ml deficiency in 85% of pregnant women with GDM and only in one out of every four pregnant women 25(OH)D deficiency in the first trimester was an independent risk factor for GDM and insulin resistance in the second trimester [25, 26]. Vitamin D deficiency is linked not only with the risk of GDM but also with the prevalence of eclampsia, anemia or infections during gestation. It also affects the term of delivery and the birth weight [27]. Vitamin D supplementation improves insulin sensitivity and glucose tolerance; however, it has not been proven that it helps to prevent GMD [28].

Vitamin D and cardiovascular disease

Accumulating data suggest that VD may play a vital role for cardiovascular disease (CVD). The first evidence of the possible link between solar UV radiation and cardiovascular mortality showing an inverse relationship between these phenomena was reported in 1981 [29]. The expression of VDR and enzymes for vitamin D metabolism has been identified in the vascular system as well as in the heart. It has been reported that VDR knockout mice suffer from cardiovascular disease and even selective VDR deletion in cardiomyocytes causes myocardial hypertrophy and exacerbates endothelial dysfunction [30, 31]. Many observational studies confirmed the association of low VD status with a higher incidence of cardiovascular events and mortality. Zitterman et al. indicated a nonlinear decline in overall mortality as 25(OH)D concentrations increase [32]. Furthermore, low 25(OH)D concentrations have been reported as an independent risk factor for cardiovascular events, in particular for sudden cardiac deaths [33]. Vitamin D deficiency has been believed to be involved in insulin resistance, type 2 diabetes, and an adverse lipid profile [34].

Bjelakovic et al. in their meta-analysis evaluated the benefits and harms of vitamin D supplementation used in primary and secondary prophylaxis of mortality. They concluded that VD seemed to decrease mortality in elderly people [35]. However, some data have provided contradictory results. Ho et al. did not confirm that low VD is a significant risk factor for the presence of either calcific atherosclerosis or obstructive coronary artery stenosis [36]. Available evidence on the effects of VD on CVD has also been inconclusive. Gepner et al. failed to confirm the improvements in central blood pressure parameters or arterial stiffness in healthy postmenopausal women during VD supplementation [37]. Other researchers also reported no effect of VD supplementation on the cardiovascular risk factors [38, 39].

Although, there is a great body of evidence that VD status may influence the risk of cardiovascular disease, final confirmation of a causal relationship between VD and the cardiovascular risk is still lacking. It is necessary to perform large randomized controlled supplementation trials with VD in specific risk groups to clarify the

role of VD in cardiovascular events. Currently available RCTs on this topic are frequently limited by the additional supplementation of calcium which may increase the risk of CVD events. At present, the data are not sufficient for general recommendations to supplement VD in order to prevent and treat CVD [39].

Vitamin D in the carcinogenesis

The non-classical effects of VD, namely anti-proliferation, pro-differentiation, immune function modulation, and anti-inflammation, have been widely debated for the past decade. In particular, a lot of attention has been paid to the potential of VD analogs alone or in combination with other anticancer agents in the treatment of a variety of cancers. The relationship between VD status and the higher incidence of many types of cancer has suggested that VD may play a role in the etiology of these forms of cancer. Based on the present findings, $1\alpha,25(OH)_2D$ may have functions beyond its well-known action on calcium homeostasis and bone mineralization. The results of many studies have corroborated the fact that $1\alpha,25(OH)_2D$ exhibits anti-proliferative, pro-differentiating, anti-inflammatory, and pro-apoptotic functions in a tissue- and cell-specific manner. What is more, it has been shown to have a growth inhibitory effect on prostate, colon, breast, lung, liver and pancreatic cancer cells which express VDR [40]. The link between VD and breast mammographic density is a controversial problem. Heo *et al.* analyzed the correlation between 25-hydroxyvitamin D (25(OH)D) level and mammographic density in healthy pre- and postmenopausal women. The results of this study showed no significant associations between serum 25(OH)D and breast density [41]. Green *et al.* found no evidence of a correlation between plasma levels VD and mammographic density after menopause but their study suggested that high mammographic density and low plasma 25(OH)D level had four times greater risk of breast cancer than low mammographic density and high serum VD [42]. In the Women's Health Initiative Calcium and Vitamin D trial the authors observed no effect of VD and calcium supplementation on mammographic density after one year follow-up [43]. Epidemiological studies have suggested that low VD levels are associated with an increased risk of breast cancer. Vitamin D receptor is a crucial mediator for the cellular effects of VD. A molecular-based epidemiological study has identified several VDR genes and the overall data showed that the FokI and BsmI gene polymorphisms significantly correlate with an increased risk of ovarian and breast cancers. Mun *et al.* claimed that VDR polymorphisms may be potential risk factors for ovarian and breast cancers. The researchers implicate that in future studies VDR genetic variation should be integrated also with a pre-diagnostic indicator of VD status [44]. Both

population and laboratory studies conducted on Polish female patients suffering from breast cancer have corroborated the important role of VD in the prevention of this disorder. An appropriate dietary intake and VD supplementation is a simple, safe and cost-effective method of reducing the risk of breast cancer development and should be recommended. The vital role of this vitamin has also been found in carcinogenesis of the ovarian cancer [45].

Vitamin D and menopausal period

The decrease in estrogens function observed during menopause, results in increased bone metabolism, a decrease in bone mineral density and the related elevated fracture risk. Furthermore, weight gain, decreased lean body mass and increased visceral fat tissue affect most women after menopause what places this group of patients at an increased risk of metabolic and cardiovascular disease. It has been well established that obesity is associated with VD deficiency [46]. This observation gives rise to the question whether VD deficiency is caused by obesity or obesity is a consequence of low VD levels. The data from the research suggest that rather obesity leads to VD deficiency, while the opposite connection seems not to be significant [47]. It has been reported that lower skin VD synthesis is observed with aging and even a similar exposure to solar radiation in elderly patients produces up to 75% less VD in comparison to young adults [48]. According to Gaugris *et al.*, the prevalence of low VD levels appears to be high in post-menopausal women, especially in those with osteoporosis and history of fracture [49]. Furthermore, PTH levels are higher in the elderly than in younger people at similar serum 25(OH)D levels, which may adversely affect the bone metabolism [50]. Additionally, the decline of estrogens after menopause decreases the activity of 1α-OHase, what results in lower synthesis of the active VD form. These results suggest that VD supplementation, even in higher doses, may be necessary in postmenopausal women to overcome high parathyroid activity, also probably exacerbated by a decreased renal function. Vitamin D supplementation seems to be the most appropriate treatment option for the population of postmenopausal patients and has been suggested by many experts as a safe and cost-effective procedure. However, the role of VD supplementation in the prevention and treatment of comorbidities associated with aging and menopausal consequences has not been completely established. Płudowski *et al.* offer elaborated consensus on supplementation guidance and population strategies for VD in Central Europe and recommend that the elderly (65 years and above) should be supplemented with 800-2,000 IU/day (20.0-50.0 µg/day) throughout the whole year, because of the reduced efficacy of VD skin synthesis. In obese el-

derly patients, supplementation of 1,600-4,000 IU/day (40-100 µg/day), depending on severity of obesity, is recommended throughout the whole year [10].

Menopausal symptoms and vaginal atrophy

Menopause is characterized by falling levels of estrogen and progesterone, which can lead to the development of symptoms including hot flushes, night sweats, and vaginal dryness. As it has already been discussed, the decline in estrogens also results in the increased bone turnover, decrease in bone mineral density and elevated risk of fracture. The quality of life may be impaired by musculoskeletal discomfort, frequent mood disturbances and an increased risk of metabolic and cardiovascular diseases. Capatina *et al.* studied VD status and biological correlations in postmenopausal women and demonstrated a widely prevalent insufficiency as only 8.1% had sufficient VD levels [51]. The Women's Health Initiative trial found borderline significant associations between 25(OH)D levels and menopausal symptoms such as sleep disturbance, emotional well-being, and energy/fatigue [52]. Most of the postmenopausal women suffer from vulvovaginal atrophy, dryness and irritation associated with estrogen deficiency. Therefore, safe treatment is desirable to enhance vaginal lubrication. Vitamin D is known to be involved in the regulation of growth and differentiation of body cells, especially squamous epithelium, present in the vagina. Therefore, this vitamin could be effective in proliferation and repair of epithelial vaginal tissue. Yildirim *et al.* have demonstrated that VD supplementation resulted in squamous maturation of the vaginal epithelium. These findings suggest that there must be an intracellular receptor in vaginal epithelium. No evidence has been found regarding the side effects of VD [53]. Rad *et al.* demonstrated that VD vaginal suppositories improve the maturation index and decrease the pH and dryness in women with vaginal atrophy due to menopause. The authors suggested that vaginal suppositories may be used as lubricants and moisturizers and they can be a new strategy of vaginal atrophy treatment in many women with contraindication to estrogen therapy which include among others the history of breast cancer, stroke and venous thromboembolism [54]. However, other studies, like the one conducted by Shirazi *et al.*, found no association between menopausal status and serum VD levels [55].

Urogenital symptoms

Only few studies in the literature have assessed the relationship between VD status and pelvic floor disorders. Parker *et al.* showed that insufficient VD is linked to a greater impact of urinary incontinence on women's quality of life [56]. Their longitudinal cohort study suggested that low intake levels of certain micronutrients may be associated with an increased risk of overactive bladder (OAB), the most significant association being with VD. Dallosso *et al.* reported that a higher VD intake significantly correlated with a reduced risk of OAB in women aged 40 and older [57]. The authors assumed that VD plays an important role in skeletal muscle efficiency and potentially in the detrusor muscle and urothelial function. Thus, they explained their observation that VD deficiency has more effects on urinary incontinence in comparison to VD sufficient women [56, 57]. Gau *et al.* reported only two cases of urgency urinary incontinence with high dose VD supplementation [58]. Vitamin D receptor has been identified in the urothelium and the smooth muscle of the detrusor wall [59]. Vitamin D insufficiency may also affect the detrusor wall contributing to symptoms of OAB and urgency urinary incontinence (UUI). Badalian *et al.* demonstrated the results of the National Health and Nutrition Examination Survey and suggested that higher VD levels are associated with a decreased risk of pelvic floor disorders in women. The authors revealed that the risk of urinary incontinence was 45% lower in people with VD levels of 30 ng/ml or higher than in those with inadequate levels [60].

Conclusions

The latest scientific findings have stressed the pleiotropic action of VD and defined the prominent role of this vitamin in the pathomechanisms of numerous disorders. The multidirectional effect of VD deficiency is present in different phases of the aging process. Its negative metabolic consequences as well as higher prevalence of cardiovascular events and increase in overall mortality points to the need to treat the deficiency or – even better – to prevent it. Randomized clinical and placebo controlled trials have found that 25(OH)D 30-80 ng/ml VD has positive effects and helps induce beneficial clinical changes. Prevention and treatment processes involve education regarding sunlight exposure and pharmacological cholecalciferol supplementation according to the recommendations for Central Europe.

Disclosure

Authors report no conflicts of interest.

References

1. Holick MF. Vitamin D deficiency. N Engl J Med 2007; 357: 266-281.
2. Adams JS, Hewison M. Update in vitamin D. J Clin Endocrinol Metab 2010; 95: 471-478.
3. Rosner CJ. Vitamin D insufficiency. N Engl J Med 2011; 364: 248-254.
4. Bener A, Al-Hamaq AO, Saleh NM. Association between vitamin D insufficiency and adverse pregnancy outcome: global comparisons. Int J Womens Health 2013; 5: 523-531.

5. Kouda K, Nakamura H, Fujita Y, et al. Vitamin D status and body fat measured by dual-energy X-ray absorptiometry in a general population of Japanese children. Nutrition 2013; 29: 1204-1208.

6. DeLuca HF. Overview of general physiologic features and functions of vitamin D. Am J Clin Nutr 2004; 80: 1689S-1696S

7. Holick MF. Vitamin D status: measurement, interpretation, and clinical application. Ann Epidemiol 2009; 19: 73-78.

8. Zitterman A. Vitamin D and disease prevention with special reference to cardiovascular disease. Prog Biophys Mol Biol 2006; 92: 39-48.

9. Dawson-Hughes B, Mithal A, Bonjour JP, et al. IOF position statement: vitamin D recommendations for older adults. Osteoporos Int 2010; 21: 1151-1154.

10. Płudowski P, Karczmarewicz E, Bayer M, et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe – recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynol Pol 2009; 60: 68-75.

11. Zerwekh JE. Blood biomarkers of vitamin D status. Am J Clin Nutr 2008; 87: 1087S-1091S.

12. Bouillon R, Van Schoor NM, Gielen E, et al. Optimal vitamin D status: a critical analysis on the basis of evidence-based medicine. J Clin Endocrinol Metab 2013; 98: 1283-1304.

13. Vimaleswaran KS, Berry DJ, Lu C, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med 2013; 10: e1001383.

14. Mutt SJ, Hyppönen E, Saarnio J, et al. Vitamin D and adipose tissue-more than storage. Front Physiol 2014; 24: 228-229.

15. Drincic AT, Armas LA, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. Obesity (Silver Spring) 2012; 20: 1444-1448.

16. Pathak K, Soares MJ, Calton EK, et al. Vitamin D supplementation and body weight status: a systematic review and meta-analysis of randomized controlled trials. Obes Rev 2014; 15: 528-537.

17. Mason C, Xiao L, Imayama I, et al. Vitamin D3 supplementation during weight loss: a double-blind randomized controlled trial. Am J Clin Nutr 2014; 99: 1015-1025.

18. Takiishi T, Gysemans C, Bouillon R, et al. Vitamin D and diabetes. Endocrinol Metab Clin North Am 2010; 39: 419-446.

19. Takiishi T, Gysemans C, Bouillon R, et al. Vitamin D and diabetes. Rheum Dis Clin North Am 2012; 38: 179-206.

20. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients 2013; 5: 2502-2521.

21. Grant WB, Holick MF. Benefits and requirements of vitamin D for optimal health: a review. Altern Med Rev 2005; 10: 94-111.

22. Sadek KM, Shaheen H. Biochemical efficacy of vitamin D in ameliorating endocrine and metabolic disorders in diabetic rats. Pharm Biol 2014; 52: 591-596.

23. Teegarden D, Donkin SS. Vitamin D: emerging new roles in insulin sensitivity. Nutr Res Rev 2009; 22: 82-92.

24. Kramer CK, Swaminathan B, Hanley AJ, et al. Prospective associations of vitamin D status with β-cell function, insulin sensitivity, and glycemia: the impact of parathyroid hormone status. Diabetes 2014; 63: 3868-3879.

25. Cho GJ, Hong SC, Oh MJ, et al. Vitamin D deficiency in gestational diabetes mellitus and the role of the placenta. Am J Obstet Gynecol 2013; 209: 560.e1-8.

26. Lacroix M, Battista MC, Doyon M, et al. Lower vitamin D levels at first trimester are associated with higher risk of developing gestational diabetes mellitus. Acta Diabetol 2014; 5: 609-616.

27. Aghajafari F, Nagulesapillai T, Ronksley PE, et al. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. BMJ 2013; 346: f1169.

28. Burris HH, Camargo CA. Vitamin D and gestational diabetes mellitus. Curr Diab Rep 2014; 14: 451.

29. Scragg R. Seasonality of cardiovascular disease mortality and the possible protective effect of ultra-violet radiation. Int J Epidemiol 1981; 10: 337-341.

30. Chen S, Law CS, Grigsby CL, et al. Cardiomyocyte-specific deletion of the vitamin D receptor gene results in cardiac hypertrophy. Circulation 2011; 124: 1838-1847.

31. Ni W, Watts SW, Ng M, et al. Elimination of vitamin D receptor in vascular endothelial cells alters vascular function. Hypertension 2014; 64: 1290-1298.

32. Zittermann A, Iodice S, Pilz S, et al. Vitamin D deficiency and mortality risk in the general population: a meta-analysis of prospective cohort studies. Am J Clin Nutr 2012; 95: 91-100.

33. Drechsler C, Pilz S, Obermayer-Pietsch B, et al. Vitamin D deficiency is associated with sudden cardiac death, combined cardiovascular events, and mortality in haemodialysis patients. Eur Heart J 2010; 31: 2253-2261.

34. Skaaby T, Husemoen LL, Pisinger C, et al. Vitamin D status and changes in cardiovascular risk factors: a prospective study of a general population. Cardiology 2012; 123: 62-70.

35. Bjelakovic G, Gluud LL, Nikolova D, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev 2014; 10: CD007470.

36. Ho JS, Cannaday JJ, Barlow CE, et al. Low 25-OH vitamin D levels are not associated with coronary artery calcium or obstructive stenoses. Coron Artery Dis 2015 [Epub ahead of print].

37. Gepner AD, Haller IV, Krueger DC, et al. A randomized controlled trial of the effects of vitamin D supplementation on arterial stiffness and aortic blood pressure in Native American women. Atherosclerosis 2015; 240: 526-528.

38. Beveridge LA, Struthers AD, Khan F, et al. Effect of Vitamin D Supplementation on Blood Pressure: A Systematic Review and Meta-analysis Incorporating Individual Patient Data. JAMA Intern Med 2015; 175: 745-754.

39. Ford JA, MacLennan GS, Avenell A, et al. Cardiovascular disease and vitamin D supplementation: trial analysis, systematic review, and meta-analysis. Am J Clin Nutr 2014; 100: 746-755.

40. van der Rhee H, Coebergh JW, de Vries E. Is prevention of cancer by sun exposure more than just the effect of vitamin D? A systematic review of epidemiological studies. Eur J Cancer 2013; 49: 1422-1436.

41. Heo DS, Lee JG, Hwang HR, et al. The association between 25-hydroxyvitamin D and mammographic density in healthy pre- and postmenopausal women regardless of the menstrual cycle phase: a cross-sectional study. Nutr Cancer 2014; 66: 97-103.

42. Green AK, Hankinson SE, Bertone-Johnson ER, et al. Mammographic density, plasma vitamin D levels and risk of breast cancer in postmenopausal women. Int J Cancer 2010; 127: 667-674.

43. Bertone-Johnson ER, Chlebowski RT, Manson JE, et al. Dietary vitamin D and calcium intake and mammographic density in postmenopausal women. Menopause 2010; 17: 1152-1160.

44. Mun MJ, Kim TH, Hwang JY, et al. Vitamin D receptor gene polymorphisms and the risk for female reproductive cancers: A meta-analysis. Maturitas 2015; 81: 256-265.

45. Walentowicz-Sadłecka M, Sadłecki P, Walentowicz P, et al. The role of vitamin D in the carcinogenesis of breast and ovarian cancer. Ginekol Pol 2013; 84: 305-308.

46. Pourshahidi LK. Vitamin D and obesity: current perspectives and future directions. Proc Nutr Soc 2014; 31: 1-10.

47. Vimaleswaran KS, Berry DJ, Lu C, et al. Causal relationship between obesity and vitamin D status: bi-directional Mendelian randomization analysis of multiple cohorts. PLoS Med 2013; 10: e1001383.

48. Hagenau T, Vest R, Gissel TN, et al. Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. Osteoporos Int 2009; 20: 133-140.

49. Gaugris S, Heaney RP, Boonen S, et al. Vitamin D inadequacy among post-menopausal women: a systematic review. QJM 2005; 98: 667-676.

50. Vieth R, Ladak Y, Walfish PG. Age-related changes in the 25-hydroxyvitamin D versus parathyroid hormone relationship suggest a different reason why older adults require more vitamin D. J Clin Endocrinol Metab 2003; 88: 185-191.

51. Capatina C, Carsote M, Caragheorgheopol A, et al. Vitamin D deficiency in postmenopausal women – biological correlates. Maedica (Buchar) 2014; 9: 316-322.

52. LeBlanc ES, Desai M, Perrin N, et al. Vitamin D levels and menopause-related symptoms. Menopause 2014; 21: 1197-1203.

53. Yildirim B, Kaleli B, Düzcan E, et al. The effects of postmenopausal Vitamin D treatment on vaginalatrophy. Maturitas 2004; 10: 334-337.

54. Rad P, Tadayon M, Abbaspour M, et al. The effect of vitamin D on vaginal atrophy in postmenopausal women. Iran J Nurs Midwifery Res 2015; 20: 211-215.
55. Shirazi L, Almquist M, Malm J, et al. Determinants of serum levels of vitamin D: a study of life-style, menopausal status, dietary intake, serum calcium, and PTH. BMC Womens Health 2013; 15: 13-33.
56. Parker-Autry CY, Markland AD, Ballard AC, et al. Vitamin D status in women with pelvic floor disorder symptoms. Int Urogynecol J 2012; 23: 1699-1705.
57. Dallosso HM, McGrother CW, Matthes RU, et al. Nutrient composition of the diet and the development of overactive bladder: a longitudinal study in women. Neurourol Urodynam 2004; 23: 204-210.
58. Gau JT. Urinary incontinence resolved after adequate vitamin D supplementation: a report of two cases. JAGS 2010; 58: 2438-2439.
59. Crescioli C, Morelli A, Adorini L, et al. Human bladder as a novel target for vitamin D receptor ligands. J Clin Endocrinol Metab 2005; 90: 962-972.
60. Badalian SS, Rosenbaum PF. Vitamin D and pelvic floor disorders in women: results from the National Health and Nutrition Examination Survey. Obstet Gynecol 2010; 115: 795-803.