

Study on serum estradiol levels in females with Vit D deficiency on Omega-3 fatty acid with vitamin D3 supplementation.

Mishra Rajiv Kumar¹, Prasad Kanhaiya²

¹Dr Rajiv Kumar Mishra (MD Medicine), Department of Medicine, SN Medical college, Agra, UP.

²Dr.Kanhaiya Prasad Professor and head Department of Medicine, Autonomous state Medical college, Kushinagar UP

Corresponding author

Dr Rajiv Kumar Mishra

(MD Medicine), Department of Medicine, SN Medical college, Agra, UP

Cite this paper as: Mishra Rajiv Kumar, Prasad Kanhaiya (2025) Study on serum estradiol levels in females with Vit D deficiency on Omega-3 fatty acid with vitamin D3 supplementation.. Journal of Neonatal Surgery, 14, (26s) 1344-1350

ABSTRACT

Results examining the impact of omega-3 fatty acids (Omega-3FA) and vitamin D3 (VD3) on serum estradiol (E2) are few and contradictory. The impact of VD3 in combination with Omega-3FA on E2 levels has not been examined in any prior research. The purpose of this study was to determine how blood E2 levels in premenopausal females with vitamin D insufficiency (VDD) were affected by VD3, Omega-3FA, and VD3 + Omega-3FA. The purpose of this study was to assess the effects of 300 mg of Omega-3FA taken daily, 50,000 IU of VD3 taken weekly, and their combination over the course of eight weeks. At eight weeks, the mid-follicular serum levels of 25-hydroxy vitamin D (25OHD) and E2 were measured. Four equal study groups were formed from a total of 128 individuals. With the exception of serum calcium ($p = 0.045$), most measures in the control group did not significantly alter, according to within-group analysis. Serum 25-hydroxyvitamin D ($p < 0.001$), serum estradiol ($p = 0.001$), serum calcium ($p < 0.001$), and serum phosphate ($p = 0.043$) all showed notable improvements in the VD group. Serum 25-hydroxyvitamin D ($p < 0.001$), estradiol ($p = 0.007$), calcium ($p = 0.003$), phosphate ($p = 0.04$), and ALT ($p = 0.035$) all showed significant changes in the Omega-3 FA group. Serum 25-hydroxyvitamin D ($p < 0.001$), estradiol ($p = 0.024$), and calcium ($p = 0.032$) all showed significant changes in the VD plus Omega-3 FA group, whereas PTH, ALT, and urea did not ($p > 0.05$). The study shows that vitamin D and omega-3 fatty acid supplements, both separately and together, were linked to significant changes in serum levels of calcium, phosphate, estradiol, and 25-hydroxyvitamin D, especially in the intervention groups. By the end of the research, parathyroid hormone levels had not changed significantly. Over the course of the study, indicators related to liver and renal function remained constant. Overall, the results show that certain biochemical indicators were improved by the interventions, and there was no indication of any negative effects on the liver or kidneys during the follow-up period

Keywords: Calcium, serum 25-hydroxyvitamin D, serum estradiol, serum calcium, and serum phosphate, Omega-3 FA.

INTRODUCTION

Omega-3 fatty acids (Omega-3FA) and vitamin D (VD) are both essential nutrients for maintaining human health¹. It is commonly known that vitamin D is crucial for bone formation, calcium and phosphate (PO₄) balance, and other bodily metabolic functions. VD deficiency (VDD) is a long-standing global problem that has been connected to a number of illnesses, such as osteoporosis in adults and rickets in youngsters². A higher risk of a number of illnesses, such as cancer, infections, autoimmune disorders, and cardiovascular diseases (CVDs), has been linked to low VD levels. However, omega-3 fatty acids are recognized as vital nutrients throughout the life cycle³. They are necessary for the development of the eyes and brain, the prevention of cardiovascular diseases, and the defense against dementia, including Alzheimer's disease. Estrogen may mediate some of the effects of VD and Omega-3FA on the human body⁴. It is well known that the VD receptor functions as a transcription factor that contributes to the production of progesterone and estradiol (E2). VDD has been linked to polycystic ovarian syndrome and impaired fertility. Furthermore, osteoporosis in postmenopausal women and bone loss in older adults are mostly caused by estrogen insufficiency and VDD⁵. Age and gonadal sex steroid hormone shortages, such as estrogen insufficiency, influence the relationship between osteoporosis and VDD in females. The impact of Omega-3FA on estrogen levels in healthy female humans has only been documented in a few number of studies⁶. According to a randomized clinical study conducted on healthy postmenopausal women, taking an Omega-3FA supplement and engaging

in moderate exercise increased estrogen levels. By altering the pro-proliferative and prosurvival effects of estrogen to a proapoptotic effect on breast cancer cells, another study clarified the possible anticancer significance of omega-3FA through its influence on estrogen signaling⁷. Research on the connection between VD and Omega-3FA and estrogen levels is necessary to further understand their roles in the human body. Research assessing the relationship between female sex hormones and VD is conflicting and inconclusive⁸. One study found a favorable correlation between testosterone and 25-hydroxy vitamin D (25OHD) levels, but not with E2 levels. However, according to a different study, there is an inverse relationship between E2 levels and 25OHD levels.

In light of the aforementioned, a convenience sample of healthy premenopausal Jordanian females with a diagnosis of VDD was used in this randomized placebo-controlled clinical study to assess the effects of taking 50,000 IU VD3 weekly, 300 mg Omega-3FA daily, and their combination for eight weeks⁹. At baseline and at the conclusion of the study (eight weeks later), fasting blood levels of 25OHD and E2 were measured.

1. MATERIALS AND METHODS

Participants in this study were invited to be a convenience sample of healthy female employees at the Autonomous State Medical College in Kushinagar, Uttar Pradesh, as well as their family members.

The ASU ethics committee gave their approval to this study in order to protect human subjects. All research subjects provided written informed permission. Every participant was made aware of the study's conditions, the advantages and disadvantages of taking the supplements, and their right to discontinue participation at any moment.

Females between the ages of 22 and 45 who had been diagnosed with VDD (VD <30 ng/mL) met the inclusion criteria. The individuals' age range was chosen to minimize the confounding effects of hormonal and behavioral changes linked to the postmenopausal stage, which usually occurs between the ages of 48 and 52. Pregnant and lactating women, women taking hormonal contraceptives, and women with a history of chronic illnesses, including renal disease (to prevent the impact of extended VD3 consumption on kidney stone formation), were excluded¹⁰. The research team contacted the females who fulfilled the inclusion criteria and asked them to come to a baseline meeting where information was gathered about their age, body mass index (BMI; kg/m²), mid-follicular E2 (pg/mL), 25OHD (ng/mL), parathyroid hormone (PTH; pg/mL), calcium (mg/dL), PO₄ (mg/dL), urea (mg/dL), and alanine aminotransferase (ALT; U/L).

This case control study involved premenopausal women with VDD who were assigned at random based on their age using stratified randomization. Patients were divided into four groups, each of which received a distinct course of treatment. Patients in Group 1 (control group) were not treated; patients in Group 2 (VD group) were given 50,000 IU of VD3 once a week; patients in Group 3 (Omega-3FA group) were given 1,000 mg of fish oil complex, which contains 300 mg of omega-3FA, once a day; and patients in Group 4 (VD plus Omega-3FA group) were given 50,000 IU of VD3 once a week in addition to 1,000 mg of fish oil complex, which contains 300 mg of Omega-3FA. The VD plus Omega-3FA group received the two supplements at intervals of four to six hours¹¹. A soft gelatin capsule containing 50,000 IU of VD3, or 1.25 mg of VD3 (cholecalciferol), was administered to the VD and VD + Omega-3FA groups. Instead of taking VD3 supplements every day, patients took them once a week to help with adherence¹².

A paramedical staff from Autonomous State Medical College Kushinagar, Uttar Pradesh, determined the therapeutic dosages of VD3 and the duration of supplement consumption based on each patient's serum 25OHD level and in compliance with the Endocrine Society's Clinical Guidelines for treating VDD in adults. The patients received safe, recommended dosages. It was discovered that exposing the entire body to midday sun for ten to fifteen minutes during the summer was equivalent to consuming up to 10,000–25,000 IU of VD3 orally. Additionally, VD3 doses comparable to those employed in this study were given over a 12-month period without causing any harm. The physiologically relevant daily DHA dosage of 722 mg is attainable by diet (about three servings of fatty fish per week). Additionally, it is comparable to dosages utilized in other child-centered studies that examined how Omega-3FA affected behavior and learning; children who took 600 mg DHA daily did not experience any negative side effects. From December 2024 to May 2025, this study was conducted at the Autonomous State Medical College in Kushinagar, Uttar Pradesh. To manage the seasonal variations in serum 25OHD levels, the study season is crucial. Sunlight is recognized to be necessary for VD synthesis. As a result, VD levels decrease and reach their lowest levels during the winter, reducing individual variances and variations.

Anthropometric measurements were taken at baseline and eight weeks beyond baseline for each of the four research groups. Fasting venous blood samples were obtained by qualified nurses during the mid-follicular phase (i.e., the first three days of menstruation) at baseline and follow-up. E2 is known to be at its lowest level during this phase, making it easier to identify changes brought on by the research intervention¹³.

Blood samples from the participants were drawn using a clot activator in 5 mL serum tubes. After being collected, the samples were centrifuged for 10 minutes at 4,000 rpm and then left at ambient temperature for 45 to 60 minutes. To quantify 25OHD, E2, calcium, serum PTH, PO₄, ALT, and urea, aliquots of at least 1 mL of serum were transferred into five labeled Eppendorf

tubes¹⁴. All samples were kept at -20°C before being examined in the Autonomous State Medical College Kushinagar, Uttar Pradesh, quality-controlled and recognized laboratories. We used chemiluminescent immunoassay technique to measure serum 25OHD levels. The Vitamin D External Quality Assessment Scheme was used to assess the method's quality.

Using a microplate reader and the Estradiol ELISA Microwells Test System, serum E2 levels were determined during the mid-follicular phase. In the follicular phase, the normal range of concentration values was 9–175 pg/mL³⁴, with a median of 48 pg/mL and a sensitivity of 8.2 pg/mL

Using a microplate reader and the PTH Intact ELISA Kit, the serum PTH levels were determined¹⁵. To quantify PTH, a two-site ELISA is employed. PTH levels in serum typically vary from 9 to 90 pg/mL. The test has a sensitivity of 1.57 pg/mL and could detect extremely low concentrations.

The CALCIUM kit was used to measure the serum calcium level. Spectrophotometry is used to measure the colorful complex that was created when calcium in the serum reacted with Arsenazo III. With a detection limit of 0.2 mg/dL, normal blood calcium levels ranged from 8.6 to 10.3 mg/dL.

Spectrophotometry was used to measure serum PO₄ levels using a Phosphorus Phosphomolybdate/UV kit. Serum PO₄ had a detection limit of 0.13 mg/dL and reference values of 2.5–4.5 mg/dL. The particular ALT kit was used to measure ALT at 340 nm. The normal range for ALT was up to 65 U/L, while the detection limit was 1.6 U/L¹⁵.

The UREA/BUN-COLOR kit was used to measure the serum urea level. At 600 nm, the absorbance was measured. 15–39 mg/dL was the standard range for serum urea levels. Since the number of eligible female candidates who would choose to participate in the study or fail to finish the various study stages was uncertain at the outset, we used a 95% confidence level and a 5% margin of error for calculating the sample size¹⁶. There were 150 female employees at ASU, our university.

The statistical software program SPSS, version 19.0 for Windows (Chicago, IL, USA), was used to conduct the statistical analysis. A paired t-test was used to compare baseline and study follow-up data (before and after the supplements (VD3, Omega-3FA, and VD + Omega-3FA) were administered) in order to identify significant changes for each study group.

Table 1- Correlation of the parameters among the groups with the mean age of 35.51±7.34

Concentrations of different parameters among different groups at baseline					
	Group-1(Control) N=32	Group 2(VD) N=32	Omega-3FA N=32	VD plus Omega-3FA group N=32	P-value
Fasting serum 25OHD (ng/mL)	10.27±4.7	10.12±7.3	22.2±12.8	11.1±3.7	P=0.001
Serum E2 mid-follicular phase (pg/mL)	87.12±24.4	65.4±6.5	54.5±15.2	41.3±2.4	P=0.000
Serum PTH (pg/mL)	13.47±7.6	26.29±8.94	27.5±14.9	16.6±7.2	P=0.001
Serum calcium (mg/dL)	11.15±1.5	13.3±1.4	16.8±3.6	10.1±1.3	P=0.000
Serum PO₄ (mg/dL)	4.23±0.42	4.6±0.6	4.21±0.8	4.6±0.7	P=0.001
ALT (U/L)	7.2±2.3	13.4±7.2	8.6±4.8	8.7±5.6	
Urea (mg/dL)	24.8±3.8	25.9±13.4	25.6±11.6	26.4±4.7	
Concentrations of different parameters among different groups at the end of study					
Fasting serum 25OHD (ng/mL)	11.23±5.6	18.25±7.6	11.4±9.2	31.4±9.6	P<0.001
Serum E2 mid-follicular phase (pg/mL)	94.14±26.4	59.4±20.6	68.3±13.7	56.5±36.5	P<0.000
Serum PTH (pg/mL)	23.16±8.4	23.02±8.18	26.6±8.1	25.9±15.9	P<0.54
Serum calcium (mg/dL)	12.18±1.1	11.8±1.3	11.3±1.9	16.9±1.3	P<0.003
Serum PO₄ (mg/dL)	4.11±0.28	3.9±0.8	3.38±0.51	4.6±0.8	P<0.000
ALT (U/L)	7.3±3.5	8.5±4.2	7.5±1.4	6.7±3.2	
Urea (mg/dL)	25.1±4.7	25.2±12.9	22.7±8.9	35.4±1.6	
Association of the p values of parameters at base line and p values at the end of study					
Fasting serum 25OHD (ng/mL)	0.25	<0.001	<0.001	<0.001	
Serum E2 mid-follicular phase (pg/mL)	0.343	0.001	0.007	0.024	
Serum PTH (pg/mL)	0.38	0.232	0.154	0.546	
Serum calcium (mg/dL)	0.045	0.000	0.003	0.032	
Serum PO₄ (mg/dL)	0.123	0.043	0.04	0.543	
ALT (U/L)	0.73	0.086	0.035	0.228	
Urea (mg/dL)	0.85	0.943	0.54	0.341	
Note: P-value for paired t-test between baseline and the end of the study for each group. Omega-3FA, omega-3 fatty acid; PO ₄ , phosphate; PTH, parathyroid hormone; VD, vitamin D.					

2. RESULTS

Table 1 presents the baseline comparison of biochemical parameters among the four study groups—Control, Vitamin D (VD), Omega-3 fatty acid (Omega-3 FA), and combination Vitamin D + Omega-3 FA groups—with 32 people in each of the 128 participants who were selected for the study. The mean \pm standard deviation is used to express the values.

The groups' fasting serum 25-hydroxyvitamin D (25-OHD) levels differed statistically significantly ($p = 0.001$). The baseline 25-OHD levels of the Omega-3 FA group were significantly higher than those of the other groups, suggesting a prevalent vitamin D deficit at baseline. The control group had the highest mean value of serum estradiol (E2), while the VD, Omega-3 FA, and VD + Omega-3 FA groups had progressively lower values ($p < 0.001$). Significant intergroup heterogeneity was also seen in serum parathyroid hormone (PTH) levels ($p = 0.001$). In contrast to the control group, the VD and Omega-3 FA groups showed higher PTH levels, which may indicate a compensatory reaction to poor vitamin D status.

The groups' serum calcium levels varied significantly ($p < 0.001$), with the Omega-3 FA group having the highest levels and the VD plus Omega-3 FA group having the lowest. Additionally, there were statistically significant differences ($p = 0.001$) in serum phosphate levels between the study groups.

Alanine aminotransferase (ALT) and urea levels did not differ statistically significantly between the groups ($p > 0.05$), suggesting similar liver and renal function at baseline. Biochemical parameters were examined between the Control, Vitamin D (VD), Omega-3 fatty acid (Omega-3 FA), and Vitamin D plus Omega-3 FA groups at the conclusion of the study period. The mean \pm standard deviation was used to express the values.

The four groups' fasting serum 25-hydroxyvitamin D levels differed statistically significantly ($p < 0.001$). At the conclusion of the study, there were also significant differences in serum estradiol levels across the groups ($p < 0.001$).

There was no statistically significant change in serum parathyroid hormone levels between groups ($p = 0.54$). There was a statistically significant difference in serum calcium levels between the groups ($p = 0.003$). Likewise, there was a significant difference ($p < 0.001$) in serum phosphate levels between the groups.

At the conclusion of the investigation, neither urea nor alanine aminotransferase levels varied statistically significantly across the study groups ($p > 0.05$). The paired t-test was used to compare biochemical parameters within the Control, Vitamin D (VD), Omega-3 fatty acid (Omega-3 FA), and Vitamin D plus Omega-3 FA groups between the beginning and completion of the study. Serum levels of 25-hydroxyvitamin D, estradiol, parathyroid hormone, phosphate, alanine aminotransferase, and urea did not alter statistically significantly in the control group ($p > 0.05$). Only the serum calcium levels showed a statistically significant change ($p = 0.045$). Serum 25-hydroxyvitamin D ($p < 0.001$), serum estradiol ($p = 0.001$), serum calcium ($p < 0.001$), and serum phosphate levels ($p = 0.043$) all showed statistically significant variations in the VD group. Serum values of urea, alanine aminotransferase, and parathyroid hormone did not show any statistically significant changes ($p > 0.05$).

Serum 25-hydroxyvitamin D ($p < 0.001$), serum estradiol ($p = 0.007$), serum calcium ($p = 0.003$), serum phosphate ($p = 0.04$), and alanine aminotransferase levels ($p = 0.035$) all showed statistically significant changes in the Omega-3 FA group. Serum urea and parathyroid hormone levels did not alter in a way that was statistically significant ($p > 0.05$).

Serum calcium levels ($p = 0.032$), serum estradiol ($p = 0.024$), and fasting serum 25-hydroxyvitamin D ($p < 0.001$) all showed statistically significant changes in the VD + Omega-3 FA group. Serum levels of urea, alanine aminotransferase, phosphate, and parathyroid hormone did not alter statistically significantly ($p > 0.05$).

3. DISCUSSION

The primary result of this innovative clinical study showed that VD-deficient females' serum levels of 25OHD were markedly raised by the combined supplementation of VD3 (50,000 IU weekly dose) and Omega-3FA (300 mg daily dose), while E2 serum levels were maintained. Another major finding of this study is the significant decrease in serum 25OHD levels at the conclusion of the study caused by Omega-3FA ($P < 0.001$). A recent study involving 134 women with oligomenorrhea revealed a negative correlation between 25OHD and E2. A similar, albeit smaller, trend in E2 levels was observed in a clinical research involving young females with high but normal 25OHD who were between the ages of 18 and 22, which is not the same age range as our study participants. This may be how VD prevents breast cancer because following four weekly doses of 28,000 IU VD3, there was a marginally significant decrease in luteal E2 levels of 3% for every 10 nmol/L increase of 25OHD. However, in postmenopausal females with typically very low E2 levels, researchers examined the association between 25OHD and sex hormones; the findings indicated that lower 25OHD was linked to lower E2 levels in females^{5&17}. Another study revealed that giving overweight postmenopausal women participating in a weight loss program 2,000 IU of VD3 per day for a year had no overall impact on lowering their E2 levels.¹⁸ However, there were larger drops in E2 levels with VD replacement, which may have had a dose-dependent effect. The flip side of the relationship between the two hormones—that is, the impact of E2 on 25OHD—was examined in females during the entire follicular phase of the menstrual cycle^{2,8,11&19}. The levels of 25OHD (total, free, bioavailable) and 25OHD binding protein did not alter in tandem with the notable shift in E2 levels at this phase, and they did not correlate with E2 at that time²⁰. Because of this, it is possible to

accurately test 25OHD at any time throughout the follicular phase of the menstrual cycle, which is another reason why the study's participants were measured at the mid-follicular phase.

There is a nonlinear inverse relationship between serum 25OHD and breast cancer risk, primarily at levels above 35 ng/mL in postmenopausal women, according to a meta-analysis of nine prospective studies on the subject^{11 & 21}. It was proposed that VD might prevent breast cancer by reducing estrogen signaling and production and downregulating the expression of estrogen receptors. Our study's findings, which showed that VD3 supplementation lowered E2 levels, somewhat supported this theory. Furthermore, research conducted both *in vitro* and *in vivo* shown that 1,25 dihydroxy VD (1,25(OH)2D) reduced estrogen signaling in breast cancer cells and inhibited the production of aromatase, an enzyme that promotes the manufacturing of estrogen from androgen. However, the opposite occurred in cultured ovarian cells, where 1,25(OH)2D accounted for 9% of the E2 production stimulation through the VD receptor, a gene transcriptional factor for the enzyme aromatase²². However, our data regarding E2 alterations during the mid-follicular phase in the liver did not conflict with the results of previous investigations. In addition to the study methodology, which includes the doses and duration of intervention, the variation in the mean age of the study participants may also be a factor in other contradictory results from earlier research when compared to our data. Recent research has focused more on the pathophysiology of age-related bone loss. It appears to start around the age of 40 and after the age of thirty. In osteoporosis, the correlation between 25OHD and E2 appears to be more pronounced^{10 & 23}. After menopause, an E2 deficit causes increased bone resorption, which increases the loss of bone mass. As a result, VDD worsens osteoporosis by reducing calcium absorption. Therefore, without having a negative impact on E2 levels, the effects of VD supplementation seen in this study may help prevent osteoporosis in early life²⁴. As a result, the combination can be suggested to enhance musculoskeletal results. The VD receptor, which functions as a transcription factor, may be responsible for modulatory effects of VD supplementation that enhance the effect of Omega-3FA on serum E2²⁵. This is because VD is hydroxylated to 25OHD before being converted to the active form, 1,25(OH) D, in the kidney by 1-hydroxylase²⁶.

Chronic kidney disease (CKD) patients may have reduced levels of 1,25(OH)2D due to decreased renal function that suppresses 1- α -hydroxylase activity^{18 & 26}. Moreover, VDD is a recognized risk factor for CVD in people with chronic kidney disease. Therefore, in order to lower the risk of CVD, it is vital to refill the levels of 1,25(OH)2D in these patients, and using Omega-3FA is one way to achieve this. Researchers showed that when dialysis patients received 2,500 mg of Omega-3FA without VD3 for six months, their levels of 1,25(OH) D dramatically increased while their levels of 25OHD remained unchanged²⁷. This action of Omega-3FA was thought to be caused by either suppression of the enzyme 24-hydroxylase, which catabolizes 1,25(OH)2D, or extrarenal 1-hydroxylase stimulation and subsequent activation of VD3. In a different study, 2,000 mg of Omega-3FA with VD3 given for three months increased (but not significantly) 1,25(OH)2D with a significant increase in 25OHD levels in eight CKD patients receiving hemodialysis and having inadequate or deficient 25OHD levels²⁸. Additionally, there was a considerable increase in the ratio of 1,25(OH)2D to 25OHD, which indicates the activation of 1-hydroxylase²⁹. One potential restriction is that we did not measure the serum levels of Omega-3FA and 1,25(OH)2D in our study groups. According to a randomized clinical research conducted on healthy postmenopausal women, taking 1,000 mg of Omega-3FA daily for four months while engaging in moderate-intensity exercise raised the levels of calcitonin, 1,25(OH)2D, and estrogen while lowering PTH³⁰. It was proposed that coordinated increases in elevated calcitonin and decreased PTH levels, which interacted to permit elevations in estrogen levels, were responsible for the elevated estrogen levels³¹. The administration of Omega-3FA to a larger number of subjects (a strong point) who were young, healthy ladies with VDD resulted in a significant rise in E2, which may be useful for treating osteoporosis in postmenopausal females³².

In many research, the combination of Omega-3FA and VD3 was assessed for a number of reasons. Among these was the VITAL research, a randomized, double-blind, placebo-controlled investigation that evaluated the impact of 1,000 mg/day of Omega-3FA and 2,000 IU/day of VD3 on the prevention of CVD and cancer in 25,875 men and women between the ages of 50 and 55^{1,5,9,11,14,17,19 & 31}.

4. CONCLUSION

The effect of the VD + Omega-3FA group combination on E2 in healthy young premenopausal females with VDD has never been demonstrated before, as far as we are aware. The reducing effects of VD alone and Omega-3FA alone on E2 and 25OHD, respectively, were reversed in the VD plus Omega-3FA group. Both 25OHD and E2 levels significantly increased in the VD plus Omega-3FA group without generating any kidney or liver damage or disruptions in PTH, calcium, or PO4 homeostasis. These results suggest that the beneficial effect of Omega-3FA on E2 by itself does not result from the reduction of 25OHD, as both E2 and 25OHD increased when combined with VD3. A number of illnesses, including but not limited to the improvement of bone-related disorders and CVDs, particularly CKD, can benefit from the use of VD with Omega-3FA. Undoubtedly, more investigation is needed to examine and validate the positive impacts of this combination..

REFERENCES

- [1] Binkley N, Gemar D, Engelke J, et al. Evaluation of ergocalciferol or cholecalciferol dosing, 1,600 IU daily or

- 50,000 IU monthly in older adults. *J Clin Endocrinol Metab.* 2011;96(4):981–988.
- [2] Clarke BL, Khosla S. Physiology of bone loss. *Radiol Clin North Am.* 2010;48(3):483–495.
- [3] Tartibian B, Hajizadeh Maleki B, Kanaley J, Sadeghi K. Long-term aerobic exercise and omega-3 supplementation modulate osteoporosis through inflammatory mechanisms in post-menopausal women: a randomized, repeated measures study. *Nutr Metab (Lond).* 2011;8(1):71–1770.
- [4] Cao W, Ma Z, Rasenick MM, Yeh S, Yu J. N-3 poly-unsaturated fatty acids shift estrogen signaling to inhibit human breast cancer cell growth. *PLoS One.* 2012;7(12):e52838.
- [5] Bloch MH, Qawasmi A. Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: systematic review and meta-analysis. *J Am Acad Child Adolesc Psychiatry.* 2011;50(10):991–1000.
- [6] Richardson AJ, Burton JR, Sewell RP, Spreckelsen TF, Montgomery P. Docosahexaenoic acid for reading, cognition and behavior in children aged 7–9 years: a randomized, controlled trial (the DOLAB Study). *PLoS One.* 2012;7(9):e43909.
- [7] Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am J Clin Nutr.* 2004;80(6 Suppl):1678S–1688S.
- [8] Ruxton CH, Reed SC, Simpson MJ, Millington KJ. The health benefits of omega-3 polyunsaturated fatty acids: a review of the evidence. *J Hum Nutr Diet.* 2004;17(5):449–459.
- [9] Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Adv Nutr.* 2012;3(1):1–7.
- [10] Lee JH, O’Keefe JH, Lavie CJ, Harris WS. Omega-3 fatty acids: cardiovascular benefits, sources and sustainability. *Nat Rev Cardiol.* 2009;6(12):753–758.
- [11] Hughes MR, Malloy PJ, Kieback DG, et al. Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. *Science.* 1988;242(4886):1702–1705.
- [12] Hilger J, Friedel A, Herr R, et al. A systematic review of vitamin D status in populations worldwide. *Br J Nutr.* 2014;111(1):23–45.
- [13] Cashman KD, Dowling KG, Škrabáková Z, et al. Vitamin D deficiency in Europe: pandemic? *Am J Clin Nutr.* 2016;103(4):1033–1044.
- [14] Morrison NA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. *Nature.* 1994;367(6460):284–287.
- [15] Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr.* 2008;87(4):1080S–1086S.
- [16] Smith TJ, Tripkovic L, Damsgaard CT, et al. Estimation of the dietary requirement for vitamin D in adolescents aged 14–18 y: a dose-response, double-blind, randomized placebo-controlled trial. *Am J Clin Nutr.* 2016;104(5):1301–1309.
- [17] Gennari C. Calcium and vitamin D nutrition and bone disease of the elderly. *Public Health Nutr.* 2001;4(2B):547–559.
- [18] Morris DH, Jones ME, Schoemaker MJ, Mcfadden E, Ashworth A, Swerdlow AJ. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. *Am J Epidemiol.* 2012;175(10):998–1005.
- [19] Zhao D, Ouyang P, de Boer IH, et al. Serum vitamin D and sex hormones levels in men and women: The Multi-Ethnic Study of Atherosclerosis (MESA). *Maturitas.* 2017;96:95–102.
- [20] Jackson RD, Lacroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. *N Engl J Med.* 2006;354(7): 669–683.
- [21] Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;96(7): 1911–1930.
- [22] Holick MF. The vitamin D epidemic and its health consequences. *J Nutr.* 2005;135(11):2739S–2748.
- [23] 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(3):746–755.
- [24] Kinuta K, Tanaka H, Moriwake T, Aya K, Kato S, Seino Y. Vitamin D is an important factor in estrogen biosynthesis of both female and male gonads. *Endocrinology.* 2000;141(4):1317–1324.
- [25] Parikh G, Varadinova M, Suwandhi P, et al. Vitamin D regulates steroidogenesis and insulin-like growth

factor binding protein-1 (IGFBP-1) production in human ovarian cells. *Horm Metab Res.* 2010;42(10): 754–757.

- [26] Grundmann M, von Versen-Höynck F. Vitamin D – roles in women’s reproductive health? *Reprod Biol Endocrinol.* 2011;9(1):146–7827.
- [27] Velija-Asimi Z. Evaluation of the association of vitamin D deficiency with gonadotropins and sex hormone in obese and non-obese women with polycystic ovary syndrome. *Med Glas (Zenica).* 2014;11(1):170–176.
- [28] Drake MT, Clarke BL, Lewiecki EM. The pathophysiology and treatment of osteoporosis. *Clin Ther.* 2015;37(8):1837–1850.
- [29] Riggs BL. The mechanisms of estrogen regulation of bone resorption. *J Clin Invest.* 2000;106(10):1203–1204.
- [30] Holick MF. Vitamin D: importance in the prevention of cancers, type 1 diabetes, heart disease, and osteoporosis. *Am J Clin Nutr.* 2004;79(3):362–371.
- [31] Zhu K, Oddy WH, Holt P, et al. Tracking of vitamin D status from childhood to early adulthood and its association with peak bone mass. *Am J Clin Nutr.* 2017;106(1):276–283.
- [32] Latimer CS, Brewer LD, Searcy JL, et al. Vitamin D prevents cognitive decline and enhances hippocampal synaptic function in aging rats. *Proc Natl Acad Sci USA.* 2014;111(41):E4359–E4366.