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Original Research

A comparison of the effect of omega-3 alone versus omega-3/ Vitamin D3 co-supplementation therapy on 25-hydroxyvitamin D levels in adults with vitamin D deficiency

Beisan A. Mohammad, Ghaleb Ali Oriquat, Heba A Khader , Husni Shukri Farah, Ahmad R. Alsayed, Jehad F. Alhmoud , Mohammad A. A Al-Najjar, Luai Hasoun, Shaimaa M. Abdelhalim, Shady Awwad, Mahmoud S. Abu-Samak

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Abstract

Background: Omega-3 fatty acid (n-3FA) supplementation may improve total hydroxyvitamin D (25OHD) levels in people with normal or low vitamin D levels. However, there was no agreement on the potential therapeutic benefit of n-3FA on vitamin D toxicity. Objective: The current randomized controlled clinical study (RCT) sought to determine if daily omega-3 fatty acid (n-3FA) supple-mentation affected 25-hydroxyvitamin D (25OHD) levels. Methods: One hundred and twenty Jordanians aged 22 to 55 were randomly allocated into three groups supplemented with the dose of 50,000 IU VD3 taken weekly (D3) alone, 300 mg n-3FA taken daily (n-3FA) alone, or their com-bination (50,000 IU VD3 taken weekly + 300 mg n-3FA taken daily (D+). In addition, fasting baseline and follow-up (ten weeks; eight weeks supplementation plus two weeks washout) of serum 25 hydroxyvitamin D (25OHD), parathyroid hormone (PTH), calcium, and leptin were assayed. Results: The n-3FA supplementation significantly decreased mean serum 25OHD levels (11.97±4.6 vs 16.5±4.8, P<0.001) compared with their peers in the D3 group who showed a significant increase in 25OHD levels (41.15±11.7 vs 19.34±6.8, P<0.001). The follow-up PTH mean levels were pro-portionally accompanied by significant alterations of 25OHD levels in the two study groups. Conclusions: This novel RCT provides that eight weeks of n-3FA therapy significantly reduced 25OHD levels, which adverse effects may accompany vitamin D status and its health-related consequences, such as bone homeostasis, cardiovascular disease, and COVID-19 conditions.

Keywords: vitamin d3; omega-3; vitamin d toxicity; cardiovascular disease; covid-19

Beisan A. MOHAMMAD*. Pharmaceutical Sciences Department- PharmD Program, Fakeeh College for Medical Sciences, Jeddah 21461, Saudi Arabia. bmohammad@fcms.edu.sa
Ghaleb Ali ORIQUAT. Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Al-Ahliyya Amman University, Amman 19328, Jordan.
Heba A Khader. Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences, The Hashemite University, PO Box 330127, Zarqa 13133, Jordan. hebaa@hu.edu.jo
Ahmad R. ALSAYED. Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University, Amman 11937, Jordan. a_alsayed@asu.edu.jo
Husni Shukri FARAH. Faculty of Allied Medical Sciences, Al-Ahliyya Amman University, Amman 19328, Jordan.
Jehad F. ALHMOUD. Department of Medical Laboratory Sciences, Jordan University of Science and Technology, P.O.Box 3030, Irbid, 22110, Jordan. jfalhmoud@just.edu.jo
Mohammad A. A AL-NAJJAR. Department of Pharmaceutical Sciences and Pharmaceutics, Applied Science Private University, Amman, Kingdom of Jordan. moh_alnajjar@asu.edu.jo
Luai HASOUN. Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University, Amman 11937, Jordan. l_hason@asu.edu.jo
Shaimaa M. Abdelhalim. Department of Pharmaceutical Sciences, Fakeeh College for Medical Sciences, Jeddah 21461, Saudi Arabia. smabdelhalim@fcms.edu.sa
Shady H. Awwad. Department of Pharmaceutical Chemistry and Pharmacognosy, Applied Science Private University, Amman 11937, Jordan. sh_awwad@asu.edu.jo
Mahmoud S. Abu-Samak*. Department of Clinical Pharmacy and Therapeutics, Faculty of Pharmacy, Applied Science Private University, Amman 11937, Jordan. m_abusamak@asu.edu.jo

INTRODUCTION

Vitamin D deficiency (VDD) is still a global problem, and the incidence of VDD is higher in Mediterranean countries such as Jordan.[1] Because of the dramatic increase in VDD prevalence among Jordanians, using 1,25-Dihydroxycholecalciferol (VD3) has increased markedly, as well as a high therapeutic dose of VD3 (50.000 IU per week) is highly recommended without medical monitoring in Jordan.[2-4] This dose is many times higher than the Recommended Dietary Allowance (RDA) for most individuals.[5] It is known that doses higher than the RDA are usually used to treat diagnosed cases of severe VDD. However, poor monitoring and follow-up of 25OHD levels may raise the likelihood of external high doses of vitamin D, causing signs of hypercalcemia, also described as vitamin D toxicity (VDT).[6]

Although VDT, usually caused by overdoses of VD3, is a rare health problem compared to VDD, it could become a severe problem due to the widespread use of VD3 at high doses, as many accidental cases were noted without reporting. In addition, hypercalcemia is the main consequence of high doses of VD3,[7] which causes calcium stone formation and other renal problems.

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Furthermore, dietary omega-3 fatty acids (n-3FA) supplementation is highly recommended for people with or susceptible to cardiovascular disease (CVD).[8] Despite the extensive use of n-3FA supplements as supportive therapy for CVD, its' independent effect on 25OHD levels has not been clarified yet. Nevertheless, most of the previous Randomized Control Trials (RCTs) were conducted to assess the monotherapy of VD3 or in combination with omega-3 on 25OHD levels. These trials revealed that the combination of VD3 with n-3FA significantly increased 25OHD levels suggesting a possible role for n-3FA during VD3 activation.[2,9] In contrast to these studies, some RCTs have shown that polyunsaturated fatty acids (PUFA), including n-3FA supplementation, may reduce the effectiveness of vitamin D or did not result in a substantial increase in the levels of 1,25(OH)2D.[2,10] Accordingly, possible adverse effects of n-3FA supplementation on 25OHD levels may opposite the goal of VD3 supplementation's inclusion, as a supportive therapy, in COVID-19 treatment protocols.

Therefore, based on the strong correlation between CVDs as a high risk of mortality with COVID-19,[11] the current RCT was designed to evaluate whether daily doses of n-3FA can affect 25OHD levels in men and women.

MATERIALS AND METHODS

Research Ethics and Participants

The Applied Science Private University (ASU) ethics committee granted ethical approval for the trials to protect human participants (Ethics approval No: DRGS-4-2018-4). The current clinical trial requirements and benefits were explained to the participants. Additionally, the risks of taking the used supplements were discussed with them. The trial's participants had the option to leave at any moment.

A random convenience sample of 157 healthy men and women aged 22 to 55 were recruited from the ASU's society and the ASU employees' relatives. Posters and other materials were distributed at the ASU faculties and centers to notify people about the research effort. The researchers discussed the project as well with the employees and instructors of ASU. Over a week, the advertisement ran throughout the university's faculties and other buildings.

As participants were being recruited, the baseline testing was completed. Ibn Al-Haytham Hospital laboratory and an internal medicine physician confirmed that participants had insufficient levels of hydroxyvitamin D (25OHD) (<30 ng/mL) and considered VDD [12]. Therefore, participants with chronic conditions such as thalassemia, cancer, osteoporosis, kidney illness, or an endocrine issue were not allowed to participate.

Randomization and Intervention

The number of participants in this RCT was consistent with those reported in comparable previous RCTs that included females with VDD.[2] As a result, approximately 30 to 40 people in each group met the inclusion requirements. Randomly participants were divided into three groups (40-44 participants per group), as demonstrated in the CONSORT flow diagram (Figure 1). The D3 group received once weekly 50,000 IU of VD3 orally (HiDee®, United Pharmaceuticals Company, Jordan) and served as a positive control group. The n-3FA group received l g oral omega-3 once daily for eight weeks (soft gelatin capsules of wild salmon and fish oil complex, Omeg-3®, Jamieson Laboratories, Canada). The equivalent of 300 mg of n-3FA, 180 mg of eicosapentaenoic acid and 120 mg of docosahexaenoic acid, are found in each capsule (DHA). The D+ group orally received both once weekly 50,000 IU of VD3 orally plus omega-3 once daily for eight weeks.

Following the Endocrine Society's Clinical Guidelines for treating VDD in adults, an orthopedic consultant from Ibn Al-Haytham Hospital determined VD3 therapeutic doses and the length of supplement intake.[13] The participants received appropriate doses that matched well within the authorized range. Similar doses of VD3 were given over 12 months without causing toxicity.[14]

The used dose of DHA (722 mg/day) in the current research could be obtained by taking three servings of fatty fish weekly. Additionally, no adverse effects were recorded associated with the 600 mg DHA daily dose. It is equivalent to doses utilized in other pediatric trials.[15]

To prevent any adverse effects from the possible accumulation of this fat-soluble vitamin, a washout period was implemented.

Data Collection Timing

This research was conducted at the Faculty of Pharmacy/ASU during the winter season (Nov/2019-Jun/2020) in Amman, Jordan. The blood sampling and data collection timing are essential to regulate the 25OHD seasonal serum level variations. In addition, it is commonly understood that sunshine is necessary for vitamin D production. As a result, levels of 25OHD decrease throughout the winter, and the lowest level can be found, which reduces variability and individual differences.

Anthropometric Measurements

Body mass index (BMI) values, height (Ht), body weight (BW), hip circumference (H), and waist circumference (WS) were taken at the study's beginning and end (8 weeks after the study's start). The ratio of hip circumference to weight (WHR) was also computed. Using electronic scales, the BW was calculated to the closest to 0.5 kg (Tanita, THD-646, Japan). Ht was measured using a vertical ruler to the nearest 0.1 cm (stadiometer). Before the measurement, the study team instructed the subjects to remove their shoes and dress in light clothing.

Outcome Measurements

Serum 25OHD Assay

The hospital's clinical laboratories routinely evaluated the serum levels of 25-hydroxyvitamin D_2 and D_3 utilizing the chemiluminescent immunoassay technique by LIAISON® 25-hydroxyvitamin D assay (DiaSorin). Magnetic particles were coated with a VD-specific antibody (solid phase), and VD was connected to an isoluminol derivative. Its intra- and inter-assay

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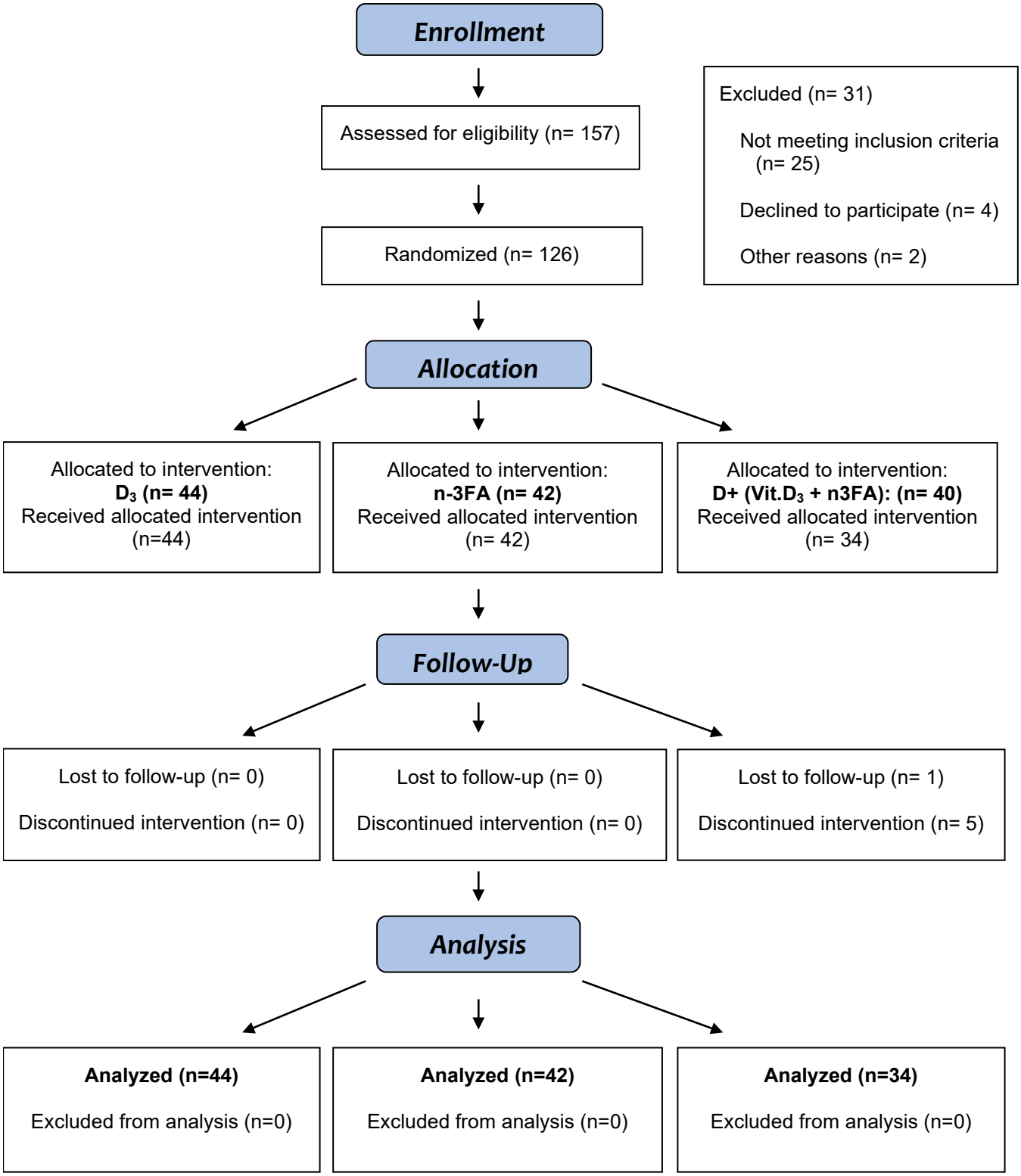

Figure 1. CONSORT flow diagram for the study, indicating the number of subjects screened, recruited and randomly assigned to the different intervention groups

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coefficients of variation were 5.0% and 8.2%, respectively. The assay's lower limit was roughly four ng/mL. The assay determines the serum 25OHD content since it has a 100% cross-reactivity with 25OHD metabolites, $25OHD_{22,}$ and $25OH_3$.

Serum PTH Assay

The serum PTH levels were measured using the PTH Intact ELISA DRG KIT and a Rayto RT2100C Microplate reader (Bio Asia Diagnostics Co Ltd, China). PTH was measured using a two-site ELISA. Serum PTH concentrations should not exceed 9–90 pg/ml. The test was 1.57 pg/ml sensitive.

Serum Calcium and Phosphate

The CALCIUM-ARSENAZO kit measured the calcium in the serum (M11570i-15, BioSystems, Spain). Using spectrophotometry, the calcium in the serum reacted with ARSENAZO III to create a colored complex (Clinical Chemistry RAL Analyzers Clima Plus, Spain). The detection limit for normal serum calcium levels is 0.2 mg/dl and ranges from 8.6 to 10.3 mg/dl. With the Phosphorus Phosphomolybdate/Uv Kit, serum PO_4 levels were evaluated (M11508i-18, BioSystems, Spain). Spectrophotometry measured serum PO_4 (Clinical Chemistry RAL Analyzers Clima Plus, Spain). The accepted value for serum PO_4 was 2.5–4.5 mg/dl, whereas the detection limit was 0.13 mg/dL.

Clinical Obesity Parameters

An enzyme immunoassay kit was used to assess the serum levels of leptin (leptin EIA - 5302, DRG Diagnostics, Germany). The triglycerides BioSystems kit measured serum triglycerides (TGs) (M11528i-20, BioSystems, Barcelona, Spain).

The fasting blood glucose (FBG) samples were processed using a Roche Cobas C501 analyzer in the clinical chemistry laboratory at the Ibn Al- Haytham Hospital (GLUC3 application, Roche, Mannheim, Germany).

Data Analysis

The SPSS version 22.0 for Windows was used for the statistical analysis (Chicago, IL, USA). First, a paired T-test (D3, n-3FA and D+) was performed to find notable variations between each trial group before and after the supplement administration. Next, a stepwise multiple linear regression test was used according to the various groups to identify potential factors that might have affected 25OHD levels at the start and end of the study. Finally, the Kolmogorov-Smirnov test was used to determine if the distribution of laboratory measurements was normally distributed.

RESULTS

Baseline characteristics of the participants

One hundred fifty-seven healthy participants enrolled in the trial, and 120 (76.4%) completed the entire trial (Figure 1, Table 1).

The baseline description of the study participants is shown in Table 2. The mean ±SD age of the participants was 37.92 ±8.3 years. All participants' baseline means 25OHD level was 17.64 ±6.7 ng/ml, ranging from 3.8 to 29.9 ng/ml. The study participants' mean baseline BMI (28.1 ±5.42 kg/m^2) indicated they were generally overweight. Other mean values of serum parameters at baseline, including fasting blood glucose (FBG), parathyroid hormone (PTH), calcium (Ca), and phosphate (PO_4) for the participants, were within the normal ranges (Table 2).

Table 1. Description of categorical variables

Variable	Categories	Frequency	Percent, %
Group	D3	34	28.3
	n-3FA	42	35
	D+	44	36.7
	Total	120	100.0
Gender	Male	56	46.7
	Female	64	53.3
	Total	120	100.0
MSEx	Yes	66	55
	No	54	45
	Total	120	100.0

D3: vitamin D3 supplemented group; n-3FA: omega-3 fatty acid supplemented group; D+: vitamin D3 + n-3FA supplemented group; MSEx, morning sun exposure.

Table 2. Baseline descriptive statistics of the fasting clinical and anthropometric parameters (n=120)

Parameter	Mean (SD)	Range	Normal Ranges
Age (years)	37.92 (8.3)	28 - 62	
25OHD (ng/mL)	17.64 (6.7)	3.8 - 29.9	30 - 50
FBG (mg/dL)	82.21(14.9)	67 - 108	70 - 110
TGs (mg/dL)	130.21 (67.7)	84 - 389	Up to 150
PTH (pg/mL)	34.43 (9.76)	9.3 – 50.1	9 - 90
Calcium (mg/dL)	9.59 (1.57)	8.30 - 10.3	8.6 - 10.3
PO_4 (mg/dL)	4.10 (0.24)	3.3-4.5	2.5 - 4.5
Body Weight (kg)	78.32 (15.7)	42 - 132	
BMI (kg/m^2)	28.1 (5.42)	17.9 – 46.8	20 - < 25
WCf (cm)	93.4 (15.12)	56-139	

25OHD: 25-hydroxy vitamin D; FBG: fasting blood glucose; TGs: Triglycerides; PTH: parathyroid hormone; PO_4: phosphate; BMI: body mass index; WCf: waist circumference.

Follow-up changes of 25OHD and associated parameters

There were no significant differences within either trial between the groups (D3, n-3FA and D+) at baseline (*p*-value >0.05 for all comparisons). Follow-up treatment of n-3FA showed a significant reduction of 25OHD levels (13.30 ±6.57 vs 18.31 ±7.35 ng/ml, *p*-value < 0.001). As expected for 25OHD levels in the D3 group, their levels were significantly increased compared with baseline levels (37.62 ±12.03 vs 15.9 ±8.1, *p*-value <0.001). The same with D+ group, the 25OHD levels were significantly increased compared with baseline levels (37.52 ±6.45 vs 16.99 ±6.36, *p*-value <0.001).

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Reverse changes in mean PTH levels accompanied significant changes in the mean 25OHD levels in both D3 and D+ groups and n-3FA group. Table 3 summarizes each treatment group's fasting serum 25OHD, PTH, calcium, and phosphate (at baseline and 10-week follow-up).

Follow-up changes of selected obesity parameters

In the n-3FA group, only the follow-up means serum leptin levels showed a significant increase from 10.92 ±7.4 to 14.71± 8.1 ng/mL, which may refer to a potential obesogenic effect of n-3FA. On the other hand, the combination therapy group (D+) caused significant increase in the TGs level after ten weeks, as shown in Table 5. However, except for BMI and body weight, D3 treatment did not significantly affect other obesity parameters, including leptin, as observed in Table 4.

Effect of n-3FA supplementation on 25OHD levels

Stepwise regression analysis was used to evaluate which independent variables (IDVs) accounted for these associations with 25OHD levels in the three study groups.

Multivariate stepwise regression analysis showed significant influences of some IDVs on serum 25OHD levels, as presented in Table 5. In the n-3FA group, baseline PTH, waist circumference, and follow-up levels of TGs were involved as the main predictors and explained ~70% of the variance in follow-up 25OHD levels (Table 5). At follow-up, significant independent variables involved in the effect of D3 supplementation on 25OHD levels were the participant's body weight, WHR, follow-up calcium levels, and morning sun exposure. Cumulatively, these variables explained 69% of the variance in follow-up 25OHD levels, as detailed in Table 5. In the D+ group, baseline participant's body weight was as the main predictor and explained ~36% of the variance in follow-up 25OHD levels (Table 5). Followed by a ~15% variance due to AST (Table 5).

DISCUSSION

The main findings revealed that a daily n-3FA supplement equivalent to 300 mg for eight weeks significantly decreased serum 25OHD levels. Furthermore, this significant reduction in the mean value of 25OHD levels was also accompanied by a significant difference between baseline and follow-up mean levels of PTH that support our findings. To our knowledge, this is the first RCT designed to examine the effect of n-3FA supplementation on the total hydroxyl vitamin D levels in humans with VDD.

These results are consistent with two recent RCTs that took up the topic as a secondary outcome and, accordingly, not directly comparable [2, 16]. Furthermore, these findings may impose a serious debate on the results of previous studies on whether n-3FA supplementations may yield supplementary impacts on circulatory 25OHD levels, including subjects with VDD. In this context, a recent systematic review of 10 RCTs by Alhabeeb

Table 3. Fasting serum 25OHD, PTH, Calcium, and Phosphate at the baseline and ten weeks follow-up of the study

		D3 Mean (SD)	n-3FA Mean (SD)	D+ Mean (SD)	F-test	*P*-value
Age	Baseline	36.2 (11.5)	35.2 (8.7)	37.7(9.4)	-0.427	*P*a =0.503
25OHD (ng/mL)	Baseline	15.9 (8.1)	18.31 (7.35)	16.99(6.36)	-1.72	*P*a = 0.0554
	Follow-up	37.62 (12.03)	13.30 (6.57)	37.52 (6.45)	86.67	*P*c < 0.001
	Change	21.73	-5.44	22.77		
	*P*a	< 0.001	< 0.001	< 0.001		
PTH (pg/mL)	Baseline	36.47 (6.1)	30.6 (9.4)	33 (12.9)	3.4	*P*a = 0.048
	Follow-up	22.46 (11.9)	30.4 (12.8)	21.3 (11.2)	14.98	*Pc < 0.001*
	Change	-10.06	-0.107	-11.63		
	*P*a	< 0.001	0.915	< 0.001		
Calcium (mg/dL)	Baseline	9.38 (1.18)	9.88 (2.29)	10.1(1.59)	0.91	*P*b = 0.368
	Follow-up	9.41 (0.9)	9.72 (0.52)	10.1 (1.4)	2.26	*P*c = 0.013
	Change	-1.60	-0.16	-0.49		
	*P*a	0.11	0.171	0.620		
PO4 (mg/dL)	Baseline	3.89 (0.4)	3.66 (0.7)	4.2 (0.3)	68.50	*P*a < 0.001
	Follow-up	3.98 (0.7)	3.93 (0.34)	4.1 (0.2)	24.73	*P*b < 0.001
	Change	-.09	-.27	-2.09		
	*P*a	0.500	<.001	0.042		

*P*a: *P*-value for paired t-test between baseline and 10 weeks follow-up of the study for each group; *P*b: *P*-value for independent t-test between baseline means of D3, n-3FA and D3 groups; *Pc*: *P*-value for independent T-test between 10 weeks follow-up means of D3, n-3FA and D3 groups. D3: vitamin D3 supplemented group; n-3FA: omega-3 fatty acid supplemented group; D+: vitamin D3 + n-3FA supplemented group; 25OHD, 25-hydroxy vitamin D; PTH: parathyroid hormone; PO_4: phosphate

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Table 4. Mean differences of selected obesity predictors at the baseline and ten weeks of study follow-up

		D3 Mean (SD)	n-3FA Mean (SD)	D+ Mean (SD)	F-test	*P*-value
Serum leptin (ng/ml)	Baseline	8.5 (5.11)	10.9 (7.4)	6.7 (4.9)	5.359	*P*b = 0.0058
	Follow-up	10.3 (7.7)	14.71(8.1)	8.1 (6.8)	8.95	*P*c = 0.0002
	Change	1.77	3.51	1.11		
	*P*a	0.085	0.0011	0.269		
BMI (kg/m^2)	Baseline	27.48 (5.2)	28.1 (5.7)	27.12 (4.2)	-0.49	*P*a = 0.633
	Follow-up	27.33 (4.9)	28 .23(5.8)	28.44 (3.7)	-0.57	*P*b = 0.572
	Change	-0.14	0.13	0.32		
	*P*a	0.038	0.266	0.333		
WS (cm)	Baseline	94.24 (12.2)	92.91 (15.3)	90.43 (16.4)	0.37	*P*a = 0.771
	Follow-up	93.35 (11.6)	93.51 (15.6)	91.45 (14.11)	0.199	*P*b =0.897
	Change	-0.89	0.55	1.01		
	*P*a	0.109	0.273	0.482		
WHR	Baseline	87.75 (11)	86 (9.8)	84.48 (12.91)	1.426	*P*a = 0.239
	Follow-up	87.47 (11.3)	86.96 (10.4)	86.5 (10.82)	0.188	*P*b = 0.904
	Change	-0.28	0.55	2.02		
	*P*a	0.542	0.449	0.282		
TGs (mg/dL)	Baseline	128.29 (55.84)	156.71 (86.34)	114.29 (57.32)	2.702	*P*a = 0.058
	Follow-up	135.41 (87.44)	168.78 (126.54)	139.11(60.71)	2.596	*P*b = 0.45
	Change	7.12	12.07	24.82		
	*P*a	0.5857	0.152	0.007		

*P*a: *P*-value for paired t-test between baseline and 10 weeks follow-up of the study for each group; *P*b: *P*-value for independent T-test between baseline means of D3, n-3FA and D+ groups; *P*c: *P*-value for independent T-test between 10 weeks follow-up means of D3, n-3FA and D+ groups. D3: vitamin D3 supplemented group; n-3FA: omega-3 fatty acid supplemented group; D+: vitamin D3 + n-3FA supplemented group; BMI: body mass index; WS: waist circumference; WHR: Waist/hip ratio; TGs: triglycerides.

Table 5. The multivariate association between study variables and follow-up serum levels of 25OHD in the participants of study groups

Dependent Variable	Study group	Univariate effect estimate	Coefficient				
			B	F	R	R2	P-value
25OHD Follow-up levels	**D_3**	BW[2]	-0.910	22.89	0.646a	0.417	<0.001
		WHR[2]	0.455	20.61	0.756b	0.571	<0.001
		Calcium[2]	0.339	17.08	0.794c	0.631	0.005
		MSEx	-0.278	15.81	0.828d	0.686	0.032
	n-3FA	PTH[1]	0.843	46.86	0.735a	0.540	<0.001
		TG[2]	-0.537	35.39	0.803b	0.645	<0.001
		WS[1]	0.212	28.74	0.833c	0.694	0.025
	D+	BW[1]	-0.674	12.85	0.599a	0.358	0.002
		AST[2]	-0.442	13.36	0.741b	0.507	0.006

et al.[17] has revealed an overall substantial increase in 25OHD levels after omega-3 intake. The review also concluded that 25OHD levels significantly increased when the intervention lasted more than eight weeks, and the baseline serum 25OHD level was less than 20 ng/ml. Moreover, omega-3 intake ≤1000 mg/day achieved higher 25OHD levels than omega-3 intake >1000 mg/day. They concluded that omega-3 supplements increased 25OHD concentrations, particularly with ≤1000 mg/day dosages and intervention durations of more than eight weeks. The effect of an interventional duration of more than eight weeks has been considered when articulating the review conclusion.

It is known that EPA and DHA are the primary marine n-3FA fatty acids in salmon. According to Malgorzata,[18] increasing fish consumption or changing its patterns might be beneficial and lead to noticeable improvements in VD status. Nevertheless, the content of VD in the wild and farmed salmonids is minimal.

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The recommended fish intakes cannot optimize the status of VD, which calls for further research to ensure the sustainable production of salmon with adequate VD.[19]

Our results also are partly consistent with other prior reports that have mentioned that omega-3 supplements did not lead to a substantial elevation in the levels of $1,25(OH)_2D$[10] or improve VD absorption[20] in kidney failure patients. Our findings can also be correlated with inferred outcomes of studies that examined the co-supplementation of VD3 and n-3FA on VD status. For example, Brennan Laing B et al.[9] used nutrient supplement capsules (Lester's Oil®) containing six ingredients, including omega-3 fish oil (DHA >255 mg, EPA >170 mg), and VD3 (500 IU astaxanthin (500 mcg)).[9] Brennan Laing B et al. observed a significant decrease in 25OHD levels, accompanied by a substantial increase in n3FA to insufficient sun exposure, not to omega-3 effects. Compared to Brennan Laing B et al.'s[9] trial, significant elevation in 25OHD levels was observed in RCTs[2,16]. These trials used small doses VD3 capsules to determine the effects of co-supplementation of omega-3 and VD3 on 25OHD levels.[2,16]

Serum levels of PTH and VD are reversely associated with seasonal changes. During summer, when serum 25OHD is high, serum PTH is low. Vice versa in the winter season.[21] as well as VDD may cause secondary hyperparathyroidism.[22] Consequently, and based on the stepwise regression, this study showed that serum PTH was the primary independent variable (R^2=0.540, Table 5) involved in the reduction of 25OHD levels in n-3FA group.

The cumulative effect of the influencing variables (PTH, TGs, and WS) explained approximately 70% of the variance in follow-up changes of 25OHD caused by daily n-3FA supplementation for eight weeks, see Table 5.

The inverse relationship between PTH and 25OHD levels in our study has previously been reported.[23] The current clinical trial showed a significant increase in serum 25OHD with decreasing PTH levels in the D3 study group. At the same time, the same association was vice versa in the n-3FA study group. Therefore, accompanying significant elevation of PTH with the reduction in 25OHD after n-3FA can be linked as shreds of evidence for the results of the current study.

On the other hand, it was revealed that VDD is more prevalent in obese people, with an inverse association between 25OHD levels and obesity markers.[24]An inverse correlation between fat mass and serum 25OHD levels might be explained by the fact that adipose tissue absorbs the fat-soluble VD.[25] In the same context, the significant increase in the mean value of leptin levels at follow-up is consistent with the assumption that obesity progression is an important mediator to explain the effect of omega on 25OHD levels.[3] Correlated between long-term supplementation of n-3FA and increased circulatory leptin levels due to increased secretion of leptin from fat cells, proportional to the amount of stored triglycerides.[26]

Though n-3FA supplementation is widely recommended for patients with heart disease to reduce the risk of sudden death,[27] consuming a high PUFA diet showed higher plasma LDL-C concentrations in a study involving two Spanish populations.[28] Thus, gene polymorphism and dose-dependent treatment protocol, as potential factors, might be involved in unexpected outcomes resulting from extensive omega-3 supplementation.

Relating to VD status, there is a positive association between VDD and hyperlipidemia.[29] Considering a low serum 25OHD level as an atherosclerosis marker since it enhances endothelial dysfunction, promotes plagues formation, and induces platelet reactivity.[29,30] Moreover, Playford et al. suggested that $1,25(OH)_2D$ serum level may be more useful in assessing the atherosclerotic risk associated with VDD.[31] Regarding the effect of VD3 supplementation, a significant reduction in LDL and TC levels was observed in different populations with VDD.[32-34] Overall, it was reported that the VD3 effect on lipid profile is influenced by baseline 25OHD level, VD3 dose used, intervention duration, participants' characteristics, and study design.[27,35] Therefore, our results seriously debate previous studies' findings on whether n-3FA supplements improve lipid profile regardless of 25OHD levels. Furthermore, no previous RCT had faithfully evaluated the supplementary effect of n-3FA on 25OHD levels in individuals with VDD. Though its originality and results were the strength of this trial, limitations included sample size and non-availability of n-3FA levels in the blood.

CONCLUSIONS

This RCT is the first study designed to assess the supplementary effects of n-3FA supplementations on 25OHD levels in people with VDD and provide that eight week of n-3FA therapy significantly declined 25OHD levels, which were accompanied by a significant increase in PTH levels. Accordingly, the intervention of n-3FA alone without VD3 co-supplementation may be accompanied by adverse effects on vitamin D status and its health-related outcomes, such as bone homeostasis, CVDs, and COVID-19 conditions.

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obtained from all subjects involved in the study.

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