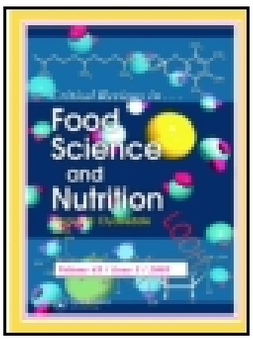

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Critical Reviews in Food Science and Nutrition

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/bfsn20

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To cite this article: Habeeb Alhabeeb, Hamed Kord-Varkaneh, Shing Cheng Tan, Mihnea-Alexandru Găman, Bander Yayha Otayf, Almuhannad Ali Qadri, Osama Alomar, Hany Salem, Ismail A. Al-Badawi & Ahmed Abu-Zaid (2020): The influence of omega-3 supplementation on vitamin D levels in humans: a systematic review and dose-response meta-analysis of randomized controlled trials, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2020.1863905

To link to this article: https://doi.org/10.1080/10408398.2020.1863905

CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION
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REVIEW

The influence of omega-3 supplementation on vitamin D levels in humans: a systematic review and dose-response meta-analysis of randomized controlled trials

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ABSTRACT

Background: Inconsistencies exist with regard to the influence of omega-3 supplementation on 25-hydroxyvitamin D (25(OH)D) levels, which could be attributed to many factors, such as the duration and dose of omega-3 supplementation, and individuals' baseline 25(OH)D levels. Therefore, to address the inconsistencies, we conducted a systematic review and dose-response meta-analysis to accurately determine the effect of omega-3 supplementation on 25(OH)D levels in humans.

Methods: We performed a comprehensive literature search in Web of Science, PubMed/Medline, Scopus, and Embase databases from inception up to January 2020. We included only randomized controlled trials (RCTs). We used weighted mean difference (WMD) with 95% confidence interval (CI) to assess the influence of omega-3 supplementation on serum 25(OH)D levels using the random-effects model.

Results: Our pooled results of 10 RCTs demonstrated an overall significant increase in 25(OH)D levels following omega-3 intake (WMD = 3.77 ng/ml, 95% CI: 1.29, 6.25). In addition, 25(OH)D levels were significantly increased when the intervention duration lasted >8 weeks and when the baseline serum 25(OH)D level was <20 ng/ml. Moreover, omega-3 intake ≤1000 mg/day resulted in higher 25(OH)D levels compared to omega-3 intake >1000 mg/day.

Conclusion: In conclusion, omega-3 supplementation increased 25(OH)D concentrations, particularly with dosages ≤1000 mg/day and intervention durations >8 weeks.

KEYWORDS
25(OH)D; Omega-3; supplementation; dose-response; meta-analysis

Introduction

Vitamin D is a fat-soluble steroid pro-hormone that is essential for bone health. It is principally synthesized in the skin after exposure to sunlight (Bikle and Christakos 2020). Once synthesized, vitamin D is transported to the liver and converted to a biologically inert form, 25-hydroxyvitamin D, abbreviated as 25(OH)D (Bikle and Christakos 2020). The 25(OH)D is subsequently converted to the active 1,25-dihydroxyvitamin D, abbreviated as $1,25(OH)_2D$, which interacts with the vitamin D receptor (VDR) to regulate calcium homeostasis (Bikle and Christakos 2020). When an individual has a low level of vitamin D, calcium absorption becomes inefficient and parathyroid hormones are released to remove excess calcium from the circulation (Goltzman, Mannstadt, and Marcocci 2018). Normally, calcium is required not only for bone mineralization, but also for various neuromuscular and metabolic activities (Wimalawansa, Razzaque, and Al-Daghri 2018). Thus, low levels of calcium as a result of vitamin D deficiency cause weakening and deformities of the bones, as well as muscle weakening (Holick 2011). In addition, the binding of vitamin D to its receptor activates a number of key genes involved in multiple biological pathways (Christakos et al. 2016). Thus, suboptimal levels of vitamin D have also been associated with the risk of developing various disorders, including but not limited to diabetes (Issa 2017; Berridge 2017), multiple sclerosis (Pierrot-Deseilligny and Souberbielle 2017), rheumatoid arthritis (Ishikawa et al. 2017), cancers (Jeon and Shin 2018; Tan 2018), and cardiovascular diseases (Podzolkov, Pokrovskaya, and Panasenko 2018).

Besides exposure to sunlight, diet is another major source of vitamin D (Nakamura et al. 2002; Macdonald 2013). However, very few natural foods are rich in vitamin D. Some examples of foods with a high content of vitamin D

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Supplemental data for this article can be accessed at https://doi.org/10.1080/10408398.2020.1863905.

include salmon, mackerel, and herring, which are also abundant in omega-3 fatty acids (Holick and Chen 2008).

Omega-3 long-chain polyunsaturated fatty acids play important roles in human health. For example, they are key components of the membranes of cells, particularly in the retina and brain, possess anti-inflammatory properties, contribute to the normal development of the fetus, and partake in the prevention of Alzheimer's disease and cardiovascular disorders (Swanson, Block, and Mousa 2012). Moreover, many studies have reported that omega-3 fatty acids may exert beneficial effects in a conundrum of disorders including cardiovascular diseases, cancers, mental illnesses (for example, depression, schizophrenia, aggression, and bipolar disorder), affections of the gastrointestinal tract (for example, Crohn's disease and ulcerative colitis), respiratory system (for example, asthma), kidneys (for example, nephropathy), and even rheumatologic afflictions (for example, systemic lupus erythematosus) (Siddiqui et al. 2004). Nevertheless, the most prominent and well-investigated effects of omega-3 are, however, related to reducing the morbidity and mortality of cardiometabolic ailments, such as diabetes, hypertension, obesity, and metabolic syndrome (Yashodhara et al. 2009).

Over the past decade, multiple reports have shown that the level of vitamin D could be influenced by omega-3 supplementation (Lee et al. 2015; Itariu et al. 2013; Al-Shaer et al. 2019, Partan et al. 2019). Nonetheless, controversies still exist regarding the impact of omega-3 supplementation on vitamin D levels in humans. For example, while Al-Shaer et al. (2019) showed that omega-3 supplementation decreased vitamin D levels, Partan et al. (2019) demonstrated that fish oil, which is the principal source of omega-3, increased vitamin D levels in humans. On the other hand, Naesgaard et al. (2017) found that omega-3 supplementation did not have any effect on the levels of vitamin D. The discrepancies in the findings of these studies might be attributed to many factors, such as the duration and dose of omega-3 supplementation, as well as the baseline vitamin D levels of the participants. In addition, individual studies typically had low sample sizes which could have limited the statistical power to detect a true impact of omega-3 on vitamin D levels. Therefore, to address the inconsistencies in the literature, we conducted a systematic review and dose-response meta-analysis to accurately determine whether omega-3 supplementation had any influence on vitamin D levels in humans.

Methods

Search strategy

This systematic review and dose-response meta-analysis was executed using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklists (Moher et al. 2015). Supplementary material, Table 1 depicts the search strategy and terms employed in our analysis. We carried out a comprehensive systematic search in the Web of Science, PubMed/Medline, Scopus, and Embase databases from inception to 28 January 2020.

Selection criteria

The following inclusion criteria were considered: studies that investigated omega-3 supplementation and were designed as randomized placebo-controlled trials (RCT); studies which provided information on the circulating 25(OH)D serum levels in the standard form (mean and standard deviation) before and after supplementation with omega-3; and studies which involved adult subjects (that is, aged $\geq$18 years). We excluded the studies that did not report 25(OH)D concentrations before and after omega-3 supplementation, papers with non-randomized study designs, animal studies, studies without a placebo group, conference abstracts, and reviews.

Data extraction

From the selected items, we extracted the following data: year of publication, data regarding the first author of the article, number of cases and controls, country, mean age of participants (years), gender of participants, length of the RCT, study design, dose of omega-3 supplementation, and the means and standard deviations of the 25(OH)D serum levels at baseline and post supplementation with omega-3. Supplementation with omega-3 consisted of various omega-3 fatty acids supplements and fish products, such as Seluang fish oil capsules (Partan et al. 2019a), 1000 mg/day of wild salmon and fish oil complex which equivalated to 300 mg/day of omega-3 fatty acids (Al-Shaer et al. 2019), 4 g/day of krill oil (Rundblad et al. 2018), 4 g/day of highly concentrated omega-3 fatty acids (Naesgaard et al. 2017), 1000 mg omega-3 fatty acids two times per day (Jamilian et al. 2017), 2.4 g/day of omega-3 fatty acids (Lee et al. 2015), 3.36 g/day long chain n-3 polyunsaturated fatty acids (Itariu et al. 2013), and omega-3 capsules (900 mg/day n-3 fatty acids) (Salari Sharif et al. 2010). Four independent researchers extracted the mentioned data and senior authors resolved the disagreements.

Quality assessment

The Cochrane Collaboration's tool with seven items (allocation concealment, random sequence generation, blinding of participants and personnel, incomplete outcome data, blinding of outcome assessment, selective reporting, and other probable sources of biases) was applied in the quality assessment of the RCTs. Each domain was scored as low, high, or unclear risk of bias. Two independent researchers evaluated the RCTs and a third reviewer resolved the disagreements.

Statistical analysis

We used weighted mean differences (WMDs) with the 95% confidence intervals (CIs) to assess the effects of omega-3 supplementation on serum 25(OH)D by using the random-effects method (derSimonian and Laird). We evaluated heterogeneity using the Q-test and the I-squared (I^2) values (P-value <0.10 and I^2 $>50\%$ indicated substantial

Table 1. Baseline characteristics of the included studies.

Author	Country (year)	Duration (months)	Age (years)	Study population	Sample size case/placebo	Omega-3 dose (mg/day)
Partan, R. U.	Indonesia (2019)	3	30	Systemic lupus erythematosus	16/16	300
Al-Shaer, A. H.	Jordan (2019)	2	32	Healthy premenopausal females with vitamin D deficiency	20/20	1000
Rundblad, A.	Norway (2019)	2	57	Healthy subjects	12/12	4000
Naesgaard, P. A.	Norway (2017)	12	61.6	Post-acute myocardial infarction	114/114	4000
Mehri Jamilian (a)	Iran (2017)	1.5	30	Gestational diabetes	35/35	2000
Mehri Jamilian (b)	Iran (2017)	1.5	30	Gestational diabetes	35/35	2000
Su Mi Lee	Korea (2015)	3	60	Patients on hemodialysis	8/7	2400
Itariu, B. K.	Austria (2013)	2	41	Severely obese, non-diabetic patients	26/28	3360
An, W. S.	Republic of Korea (2012)	6	56	Patients on hemodialysis	23/20	3000
Salari Sharif, P.	Iran (2010)	6	60	Postmenopausal osteoporotic women	13/12	900

heterogeneity). We performed a stratified analysis to identify the source of heterogeneity among the analyzed trials. We ascertained publication bias using the Egger's and Begg's statistics as well as funnel plot inspection (defined as significant if the P-value was <0.05). The probable effects of omega-3 dosage (mg/day), intervention duration (weeks), and baseline serum 25(OH)D (ng/ml) were measured using fractional polynomial modeling in meta-regression analysis and non-linear dose-response meta-analysis. All the statistical tests were executed using the Stata software version 14 (Stata Corp. College Station, Texas, USA) and a P-value < 0.05 was reported as statistically significant.

Results

The primary systematic search identified 1494 studies from PubMed/Medline, Web of Science, Scopus, and Embase databases (Supplementary material, Figure 1). Duplicate studies were removed and 1029 studies remained. During the primary screening, which was based on the review of study titles and abstracts, 941 studies were excluded and 88 studies remained for full-text examination. During the secondary screening, 79 studies were excluded for the following reasons: non-RCT study design, studies reported duplicate data, and studies did not include data of interest. Finally, nine RCTs comprising 10 study arms were included in the quantitative meta-analysis (Partan et al. 2019; Al-Shaer et al. 2019; Rundblad et al. 2018; Naesgaard et al. 2017; Jamilian et al. 2017; Lee et al. 2015; An et al. 2014; Itariu et al. 2013; Salari Sharif et al. 2010).

Study characteristics

The characteristics of the eligible studies are presented in Table 1. The dose of omega-3 intake ranged between 300 and 4000 mg/day. We included one study performed in Indonesia (Partan et al. 2019), one in Jordan (Al-Shaer et al. 2019), two in Norway (Rundblad et al. 2018; Naesgaard et al. 2017), two in Iran (Jamilian et al. 2017; Salari Sharif et al. 2010), one in Korea (Lee et al. 2015), one in Austria (Itariu et al. 2013), and one in the Republic of Korea (An et al. 2014). All the studies were published between 2010 and 2019. All studies were RCTs. The median duration of the studies was 8 weeks. The studies were conducted on different populations, including healthy subjects (Rundblad et al. 2018; Al-Shaer et al. 2019; Itariu et al. 2013), patients with systemic lupus erythematosus (Partan et al. 2019), patients with acute myocardial infarction (Naesgaard et al. 2017), patients with gestational diabetes (Jamilian et al. 2017), patients undergoing hemodialysis (Lee et al. 2015; An et al. 2014), and women with osteoporosis (Salari Sharif et al. 2010).

Quality assessment

Supplementary material, Figure 2 shows the risk of bias summary and graph of the included studies. Two studies did not report the details of randomization process; hence, we scored the random sequence generation domain as unclear risk. Four studies did not provide sufficient information about allocation; hence, we scored the allocation domain as unclear risk. In one study, the allocation was known to the investigators; hence, we scored the allocation concealment domain as high risk. Two studies were designed as open-label RCTs; hence, we scored the performance bias domain as high risk. Two studies did not mention if the RCTs were registered; hence, we scored the reporting bias domain as unclear risk. All other domains showed low risk.

Meta-analysis results

A total of 10 studies comprising 601 individuals (omega-3 = 302, placebo = 299) reported changes in 25(OH)D serum plasma concentrations as an outcome measure. Pooled results using the random-effects model demonstrated an overall significant increase in 25(OH)D level following omega-3 intake (WMD = 3.78 ng/ml, 95% CI: 1.29, 6.25, $P = 0.003$; Figure 1). However, a significant degree of heterogeneity among the studies was observed ($P < 0.001$, $I^2 = 99\%$).

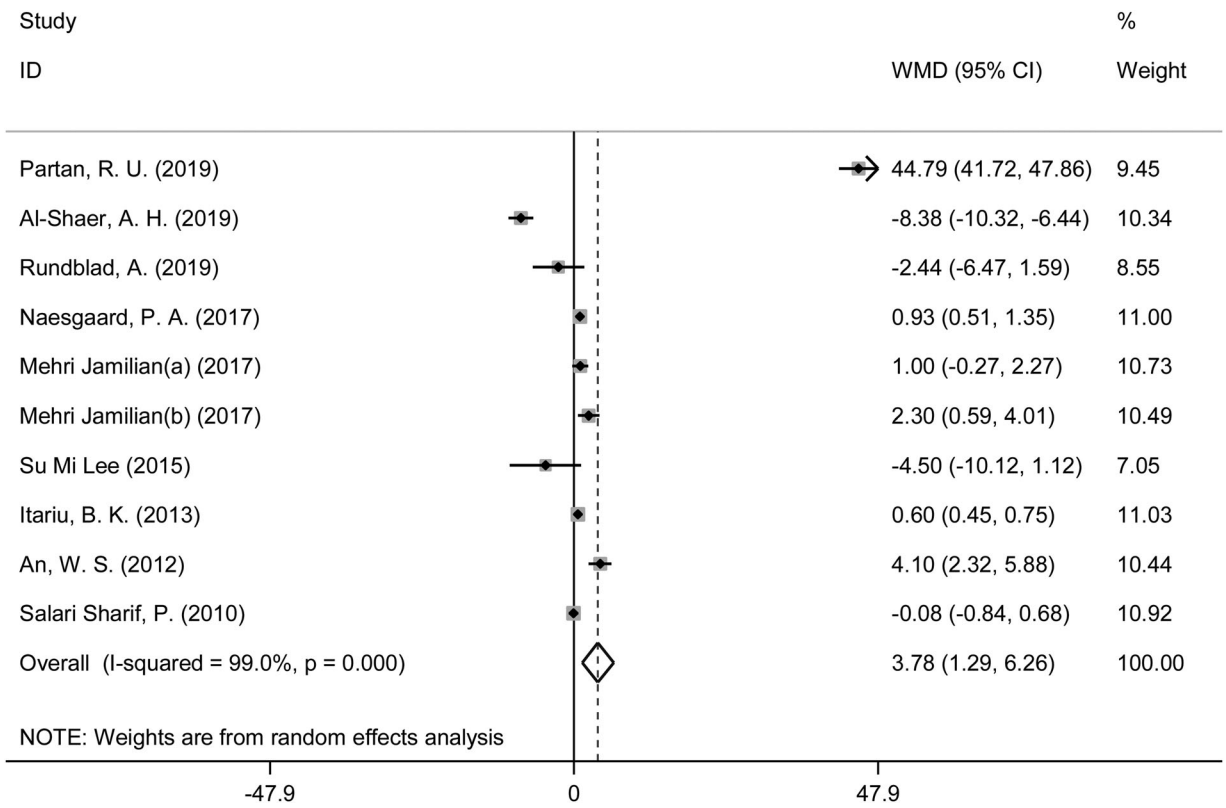

Figure 1. Forest plot of randomized controlled trials investigating the effects of omega-3 supplementation on 25(OH)D level.

Subgroup analysis

We subsequently stratified the studies based on the omega-3 dosage, intervention duration (weeks), and baseline serum 25(OH)D level (ng/ml) (Table 2). These analyses could not identify any source of heterogeneity. In addition, 25(OH)D levels significantly increased when the intervention duration lasted >8 weeks (WMD = 9.11 ng/ml, 95% CI: 2.09, 16.13) and when the baseline serum 25(OH)D level was <20 ng/ml (WMD = 1.48 ng/ml, 95% CI: 0.05, 2.92). Moreover, omega-3 intake ≤1000 mg/day resulted in a greater increase in 25(OH)D levels (WMD = 1.24 ng/ml, 95% CI: 0.66, 1.81) when compared to omega-3 intake >1000 mg/day (WMD = 0.64 ng/ml, 95% CI: 0.51, 0.78).

Dose-response and meta-regression analyses

Subsequent analysis of the relationship between baseline serum 25(OH)D with serum 25(OH)D alterations revealed a positive correlation in a non-linear dose-response analysis (coefficient = 0.68, $P = 0.002$) and linear meta-regression analysis (coefficient = 1.02, $P = 0.018$). Significant associations were not observed for the other outcomes pertaining to omega-3 dosage and intervention duration (Figure 2).

Publication bias and sensitivity analysis

By applying the Begg's and Egger's tests, we did not find a significant publication bias among the included studies ($P = 0.531$ and $P = 0.475$, respectively). Moreover, visual inspection of the funnel plot illustrated no evidence of publication bias (Supplementary material, Figure 3). To gauge the impact of each single study on the combined effect size, we removed each trial at a time from the analysis and accounted for their individuality. We observed no significant effects of any individual study on the combined effect size.

Discussion

Vitamin D is a fat-soluble compound which acts as a precursor of calcitriol, a steroid hormone regarded as an essential element in the normal health of the bones and in a normal functioning of the immune system (Wu et al. 2019). Vitamin D also plays a leading role in the differentiation of the epidermis, hair follicle cycling and growth, healing of wounds, and the normal activity of the body's barriers against pathogens. Also, it has been hypothesized that vitamin D possesses antineoplastic, anti-psoriasis, anti-metabolic syndrome, and anti-oxidant properties. Moreover, it has been proposed that vitamin D can modify the epigenome and serve in the future as an epigenetic agent (Bikle and Christakos 2020; Stanescu et al. 2018; Nur et al. 2020; Găman, Egbuna, and Găman 2020).

Considering an optimal level of 25(OH)D is necessary to maintain the homeostasis of calcium and phosphate in the body, investigating therapeutic strategies to maintain a normal concentration of vitamin D has become the priority of a myriad of studies. Thus, we aimed to unravel what was the effect of omega-3 supplementation on 25(OH)D levels, taking into consideration that omega-3 supplements have been prescribed for a long time due to their potential

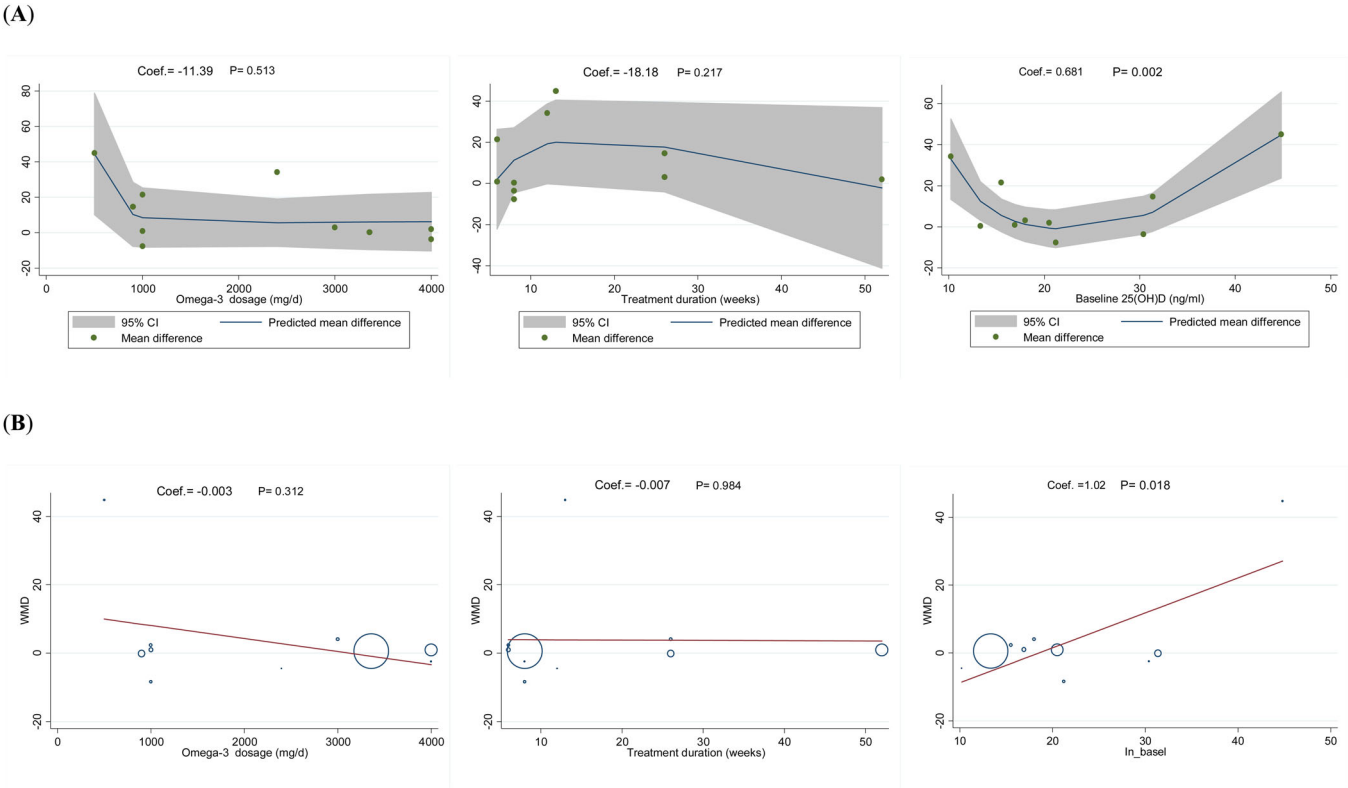

Figure 2. Dose-response analysis (A) between serum 25(OH)D changes and omega-3 dosage (mg/day), intervention duration (weeks), and baseline serum 25(OH)D level (ng/ml). Meta-regression analysis (B) between serum 25(OH)D changes and omega-3 dosage (mg/day), intervention duration (weeks), and baseline serum 25(OH)D level (ng/ml).

Table 2. Subgroup analysis showing the pooled estimates of the effects on 25(OH)D level according to omega-3 intake dosage, intervention duration, and baseline 25(OH)D level.

Group	*n*	WMD (95% CI)	*P*-value	*P*-heterogeneity	I^2 (%)
Omega-3 intake dosage (mg/day)					
≤1000	5	1.24 (0.66 to 1.81)	<0.001	<0.001	99
>1000	5	0.64 (0.51 to 0.78)	<0.001	<0.001	81
Intervention duration (weeks)					
≤8	5	−1.23 (−3.97 to 1.49)	0.38	<0.001	95
>8	5	9.11 (2.09 to 16.13)	0.01	<0.001	99
Baseline 25(OH)D (ng/ml)					
<20	5	1.48 (0.05 to 2.92)	0.04	<0.001	81
≥20	5	6.85 (−0.49 to 14.21)	0.06	<0.001	99

cardiovascular benefits. Omega-3 polyunsaturated fatty acids, which can be found in a variety of fish and fish products (for example, salmon, mackerel, herring, or phytoplankton), possess anti-arrhythmic, anti-thrombotic, blood pressure-lowering, lipid-lowering, anti-inflammatory, and anti-oxidative stress properties, and have been employed in the clinical practice with the aim of reducing the burden of cardiovascular disorders worldwide (Rimm et al. 2018; Mori 2017).

Our systematic review and meta-analysis regarding the effects of omega-3 supplementation on 25(OH)D levels analyzed nine RCTs, comprising 10 study groups with a total of 601 participants. We reported an overall significant increase in 25(OH)D following omega-3 supplementation in humans (WMD = 3.77 ng/ml, 95% CI: 1.29, 6.25, $P = 0.003$). Interestingly, omega-3 supplementation increased 25(OH)D levels irrespective of the amount prescribed, but was more pronounced when the dose of omega-3 was <1000 mg/day (WMD =1.24 ng/ml), although a dose ≥1000 mg/day also led to an increase in vitamin D levels (WMD = 0.64 ng/ml). Supplementation with omega-3 polyunsaturated fatty acids has been the focus of attention of many researchers in the recent years, mainly because omega-3 polyunsaturated fatty acids are known to possess cardio-protective effects which have been exploited to reduce the burden of cardiovascular disease worldwide (Radcliffe et al. 2016). However, there is a current gap of knowledge in the literature regarding which doses of omega-3 supplements are safe and which can potentially be harmful in humans. The relationship between vitamin D and omega-3 fatty acids is far from clear, with both substances also possessing potential harmful effects on human health. For example, some supplements containing omega-3 fatty acids can lead to drug-drug interactions with anticoagulants, and some can lead to intoxications with heavy metals, especially methyl-mercury, due to the contamination of fish and fish products with such substances (Güttler et al. 2012). Vitamin D toxicity includes, on the

other hand, kidney damage and an increased risk of hypercalcemia (Güttler et al. 2012). The dose-dependent effect of omega-3 on vitamin D levels could probably be explained by the fact that once the deposits of vitamin D are replaced, the body begins to discard the excessive amounts of omega-3, which are no longer needed in the vitamin D metabolism and homeostasis. Thus, the body prevents any potential toxic effects of increased omega-3 levels, as well as an excessive production of vitamin D when seen as unnecessary. Moreover, cessation of supplementation is associated with a reduction in the concentration of the supplemented substance. For example, Rahesh et al. (2020) have reported a decrease in vitamin D and calcium in the urine in a patient with hypervitaminosis D who had consumed supplements unregulated by the Food and Drug Administration (FDA) and experienced nontoxic hypervitaminosis D (Rahesh, Chu, and Peiris 2020). Similarly, the same mechanisms can apply to omega-3 fatty acids taken as supplements. Moreover, the increase in vitamin D levels might also be related to the reno-protective effects on omega-3 fatty acids. In a recent publication, omega-3 fatty acids have demonstrated reno-protective actions in a murine model of gentamicin-induced nephrotoxicity. Fish oil, which is rich in omega-3 fatty acids, led to a reduction in creatinine and blood urea nitrogen in the serum, as well as excretion of albumin into the urine (EL-Ashmawy et al. 2018). However, data from the VITamin D and OmegA-3 TriaL (VITAL) have suggested that neither supplementation with vitamin D or omega-3 fatty acids results in a decline in the incidence of cardiovascular disorders or invasive malignancies (Manson et al. 2019a; Manson et al. 2019b). Thus, further research is needed to investigate the effects of these supplementation on human health.

Unsurprisingly, the time needed for omega-3 supplementation to produce effects on vitamin D levels exceeded 8 weeks of administration. A duration of administration shorter than 8 weeks did not lead to a significant increase in vitamin D (WMD = -1.23 ng/ml), but showed a tendency to reduce vitamin D levels. However, a prescription which lasted longer than 8 weeks led to a significant elevation in vitamin D levels (WMD = 9.11 ng/ml). This finding comes as no surprise, since the administration of any drug takes time to produce effects. For example, Skulas-Ray also reported that an intervention duration of 8 weeks was needed for omega-3 fatty acids supplements to lower triglycerides in subjects suffering from hypertriglyceridemia (Skulas-Ray et al. 2011). Interestingly, the levels of vitamin D in our body might be in fact genetically determined. According to a recent genome-wide association study, 69 independent loci were identified, of which 63 were not related to the canonical metabolic pathway involved in vitamin D homeostasis. Also, social and economic factors seem to influence this process as well, probably due to inter-human differences related to sun exposure (Manousaki et al. 2020). Also, the time-dependent response to omega-3 fatty acids supplementation might be related to the parathyroid hormone type 1 receptor whose activation depends on extracellular ligand-regulated kinases (ERK). Studies have shown that ERK are intensely phosphorylated by long chain polyunsaturated fatty acids, namely docosahexaenoic and eicosapentaenoic acids (Candelario, Tavakoli, and Chachisvilis 2012).

In our meta-analysis, omega-3 supplementation led to a significant increase in vitamin D levels in subjects with a baseline 25(OH)D <20 ng/ml (WMD = 1.48 ng/ml, 95% CI: 0.05, 2.92; $P = 0.04$) and not in subjects with a baseline 25(OH)D ≥20 ng/ml (WMD = 6.85 ng/ml, 95% CI: −0.49, 14.21; $P = 0.06$). Subjects with a level of 25(OH)D below 20 ng/mL are considered vitamin D-deficient and patients with a level of vitamin D between 21 and 29 ng/ml are regarded vitamin D-insufficient. Subjects who have a vitamin D level >30 ng/ml at lab tests have normal levels of 25(OH)D (Güttler et al. 2012). This is another argument that the relationship between omega-3 fatty acids and vitamin D levels is intricate. However, our systematic review and meta-analysis contributed to fulfilling this gap of knowledge in the literature, since most studies exploring omega-3 fatty acids and vitamin D supplementation on the human health were executed as co-interventions (that is, vitamin D plus omega-3 fatty acids). Thus, it was difficult to assess whether the effects of these substances were synergistic, or whether their co-prescription influenced their actions. Vitamin D deficiency and vitamin D insufficiency are serious threats to the global public health, with >1 billion people suffering from these conditions worldwide. Addressing these aspects remains an important concern in our clinical practice, and we must take into consideration that vitamin D deficiency could have been involved in the development of several conditions for which patients received omega-3 supplements in order to raise their vitamin D levels (Holick 2017). For example, inadequate levels of vitamin D have been recorded in systemic lupus erythematosus, gestational diabetes, obesity, osteoporosis, chronic kidney disease patients on hemodialysis, and other conditions (Islam et al. 2019; Rizzo et al. 2019; Rajput et al. 2019; Shulhai, Pavlyshyn, and Shulhai 2019; Zhang et al. 2019; Memon, Alam, and Iftikhar 2020).

Our systematic review and meta-analysis has some important strengths. To our knowledge, this is the first systematic review and meta-analysis to explore the effect of omega-3 fatty acids supplementation on vitamin D levels. Since most studies conducted so far have explored the co-supplementation of omega-3 fatty acids and vitamin D, our report has identified that omega-3 also influences the levels of vitamin D in the body. Thus, from a clinical implication perspective, when omega-3 fatty acids and vitamin D are co-prescribed, a reduction in the dose of vitamin D may be necessary to prevent potential vitamin D-induced adverse effects. We were also able to synthesize data from several RCTs conducted in participants with various health ailments. However, our study also has some limitations. One important limitation is the heterogeneity of the selected articles, which involved subjects with a myriad of disorders, thus making it difficult to assess if the effect of omega-3 fatty acid supplementation on vitamin D levels remains the same across a large array of health diseases. Another

limitation of our study would be that we were unable to assess the dietary intake of omega-3 in the subjects included in the analysis before the intervention.

In conclusion, the main results of this research indicate a significant increase in 25(OH)D level following omega-3 supplementation. Additionally, omega-3 dosages of <1000 mg/day and intervention durations of >8 weeks significantly increased 25(OH)D levels.

Disclosure statement

All the authors declare no conflicts of interest.

Funding

This research did not receive any specific grant from funding agencies.

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