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Clinical Trial

Effects of vitamin D3, omega-3s, and a simple strength training exercise program on bone health: the DO-HEALTH randomized controlled trial

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Abstract

Evidence on the effects of Vitamin D, omega-3s, and exercise on areal bone mineral density (aBMD) in healthy older adults is limited. We examined whether vitamin D3, omega-3s, or a simple home-based exercise program (SHEP), alone or in combination, over 3 years, improve lumbar spine (LS), femoral neck (FN), or total hip (TH) aBMD assessed by DXA. Areal BMD was a secondary outcome in DO-HEALTH, a 3-year, multicenter, double-blind, randomized $2 \times 2 \times 2$ factorial design trial in generally healthy older adults age ≥ 70 years. The study interventions were vitamin D3 (2000IU/d), omega-3s (1 g/d), and SHEP (3×30 min/wk), applied alone or in combination in eight treatment arms. Mixed effects models were used, adjusting for age, sex, BMI, prior fall, study site, and baseline level of the outcome. Main effects were assessed in the absence of an interaction between the interventions. Subgroup analyses by age, sex, physical activity level, dietary calcium intake, serum 25(OH)D levels, and fracture history were conducted. DXA scans were available for 1493 participants (mean age 75 years; 80.4% were physically active, 44% had 25(OH)D levels <20 ng/mL). At the LS and FN sites, none of the treatments showed a benefit. At the TH, vitamin D versus no vitamin D treatment showed a significant benefit across 3 years (difference in adjusted means [AM]: 0.0035 [95% CI, 0.0011, 0.0059] g/cm). Furthermore, there was a benefit for vitamin D versus no vitamin D treatment on LS aBMD in the male subgroup (interaction $P = .003$; ΔAM: 0.0070 [95% CI, 0.0007, 0.0132] g/cm). Omega-3s and SHEP had no benefit on aBMD in healthy, active, and largely vitamin D replete older adults. Our study suggests a small benefit of 2000 IU vitamin D daily on TH aBMD overall and LS aBMD among men; however, effect sizes were very modest and the clinical impact of these findings is unclear.

Keywords: aging, DXA, exercise, nutrition, osteoporosis

Lay Summary

Vitamin D, omega-3 fatty acids (omega-3s), and strength training are simple but promising strategies to improve bone health; however, their effect in healthy older adults over a period of 3 years was unclear. In this study, we examined whether daily vitamin D supplementation (2000 IU/d), daily omega-3s supplementation (1 g/d), or a simple strength training program performed 3 times per week, either applied alone (eg, only vitamin D supplements) or in combination (eg, vitamin D and omega-3s supplements) could improve bone density at the spine, hip, or femoral neck. We included 1493 healthy older adults from Switzerland, Germany, France, and Portugal who were at least 70 years of age and who had not experienced any major health events in the 5 years before study start. Taking omega-3s supplements showed no benefit for bone density. Similarly, the simple strength exercise program showed no benefit. In contrast, participants receiving daily vitamin D supplements experienced a benefit at the hip. However, it should be noted that the effect across 3 years was very small.

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Introduction

Osteoporosis is a progressive age-related condition characterized by loss of bone mass and deterioration of tissue microarchitecture,[1,2] resulting in an increased risk of fractures and associated personal, societal, and economic burden.[2,3] In 2019, ~4.3 million new osteoporotic fractures occurred in Europe, which is equivalent to eight new fractures every minute.[2] Osteoporotic fractures are associated with significant disability such as mobility impairment, chronic pain, loss of independence, and premature mortality.[3–5]

The primary goal of osteoporosis therapy is the prevention of fractures and should be aimed at modifiable risk factors, such as improving indices of bone strength. DXA-derived areal bone mineral density (aBMD) is the gold standard to measure changes in bone mass in clinical practice.[6] Areal BMD is a useful measure to monitor treatment response and assess fracture risk,[7,8] accounting for 60–70% of bone strength.[9] Quantification of other indices of bone strength, such as trabecular architecture estimated by trabecular bone score (TBS), can provide additional important information about mechanical resistance to fracture beyond aBMD.[8,9]

Supplementation with vitamin D or omega-3 fatty acids (omega-3s), and exercise have been suggested as public health strategies to increase bone health and consequently reduce fracture risk. Vitamin D exerts its effects on bone through the vitamin D receptor, which is expressed in osteoblasts,[10] and through intestinal calcium absorption.[11] The evidence on the effects of Vitamin D supplements in primary osteoporosis prevention is controversial.[12,13,14,15] Furthermore, inconsistent findings have been attributed to variation in dosing regimens (eg, daily vs. bolus dosing,[16] high vs. standard dose), co-administration of calcium supplements,[17,18] and limitations in trial designs (eg, short follow-up, small sample size).[14] The DO-HEALTH trial was specifically designed as a primary prevention trial[19] to contribute evidence on the effect of vitamin D on bone in generally healthy and active older adults, unselected for risk of vitamin D deficiency or osteoporosis.

Mechanistically, omega-3s may affect bone health by decreasing the levels of pro-inflammatory cytokines involved in the regulation of bone turnover and through up-regulation of vitamin-D–dependent intestinal calcium absorption.[20] Data from observational studies suggest an association between higher omega-3s intake and maintenance of femoral neck aBMD[21] and 45% reduced hip fracture risk,[20] compared with low intake. However, data from clinical trials on the effect of omega-3s on aBMD[22,23] are limited.

Exercise regulates bone remodeling through mechanotransduction, a process during which osteocytes sense and transform mechanical signals into intercellular signals which regulate bone turnover.[24] In addition, exercise can exert beneficial effects on bone through indirect pathways, for example through cytokines (eg, anti-inflammatory) or myokines.[24] The benefits of exercise on spine and proximal femur aBMD have been well documented, particularly for strength and resistance training interventions.[25,26]

Given the distinct mechanistic pathways and relevance of vitamin D, omega-3s, and exercise for bone health, we hypothesized that the combination of these interventions may exhibit greater benefits than either alone. However, this hypothesis could not be confirmed for the primary outcome non-vertebral fracture risk in DO-HEALTH, where none of the interventions had a benefit.[27] Of note, the fracture rate in DO-HEALTH was lower than expected. The effects of vitamin D and omega-3s supplementation on fracture risk (any fracture, non-vertebral fractures, hip fractures) was also examined in the US VITAL trial, which, in line with DO-HEALTH, found no benefit for vitamin D and omega-3s (same doses as in DO-HEALTH) in 25 871 generally healthy adults age 50 years and older followed for a median of 5.3 years.[28,29]

In this analysis, we examine the individual and combined effects of vitamin D, omega-3s, and a simple home strength exercise program on aBMD, a predefined secondary outcome in DO-HEALTH, assessed in 1493 of 1503 DO-HEALTH participants recruited at four of seven recruitment sites. Furthermore, we explored treatment effects on TBS.

Materials and methods

Study design

DO-HEALTH was a double-blind, randomized controlled, $2 \times 2 \times 2$ factorial design trial, designed to investigate six primary outcomes of healthy aging (diastolic and systolic blood pressure, Short Physical Performance Battery score, Montreal Cognitive Assessment Score, incidence of non-vertebral fractures and infections over 3 years). It included 2157 generally healthy older adults from seven study centers in five European countries.[27] DXA-derived aBMD at the lumbar spine (LS), femoral neck (FN), and total hip (TH) were predefined secondary outcomes in DO-HEALTH and measured in participants from four study centers, which were equipped with DXA scanners (Zurich, Switzerland; Berlin, Germany; Toulouse, France; Coimbra, Portugal). TBS was included as an additional analysis, which was not predefined. The detailed study protocol and statistical analysis plan have been published.[19]

The trial protocol was approved by regulatory agencies of all countries[19] and was registered in the International Trials Registry (clinicaltrials.gov, registration ID: NCT01745263) and the European Union Clinical Trials Register (Registration ID: 2012–001249-41). Approval for TBS analyses was obtained after completion of the trial from the ethics committee in Zurich (2023-01084).

Study participants

Participants had to be ≥70 years of age, sufficiently mobile to come to the study center, living in the community, and score at least 24 points on the Mini Mental State Examination to be eligible for the trial. Exclusion criteria relevant to bone health and fracture risk included major health events in the 5 years prior to enrolment (eg, cancer, myocardial infarction, stroke), use of active vitamin D metabolites, parathyroid hormone (PTH) treatment (eg, teriparatide) or calcitonin, osteodystrophia deformans (Paget's disease), hypo- or hyperparathyroidism, and epilepsy or use of anti-convulsive drugs. Furthermore, participants had to be willing to limit calcium supplementation to a maximum dose of 500 mg/d, limit vitamin D supplementation to 800 IU/d, and forgo omega-3s supplementation for the duration of the 3-year trial. Participants with acute fractures within the past 6 weeks were only temporarily excluded and eligible after fracture healing. The full list of eligibility criteria has been published elsewhere.[19]

Randomization and blinding

Participants were allocated to one of the eight treatment groups using computer-based (DO-HEALTH randomization software) stratified block (block size of 16 individuals)

randomization. Stratification was based on age (70–84 years, ≥85 years), sex, study center, and history of falling in the 12 months prior to enrolment (yes/no). Treatment allocation was concealed to investigators and participants. Qualified and trained study staff performed all assessments and examinations, according to standard operation procedures. All study staff, data analysts, and participants were blinded to group allocation, except for a physiotherapist who provided the instructions for the home exercise programs but was otherwise not involved in the trial.

Interventions

Vitamin D capsules contained 1000 IU of vitamin D3 (cholecalciferol), stabilized with dl-α-tocopherol (vitamin E, 2.5 permille). Placebo capsules contained high oleic sunflower oil and were identical to vitamin D3 capsules in appearance. Participants were instructed to take two capsules per day, for a total dose of 2000 IU vitamin or placebo.

Omega-3s capsules contained 500 mg of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in a ratio of 1:2. Placebo capsules contained high oleic sunflower oil and were identical with vitamin D3 capsules in appearance. Participants were instructed to take two capsules per day, for a total dose of 1000 mg of EPA and DHA, or placebo.

The simple home exercise program (SHEP) was a strength-training program which consisted of the following five exercises: sit-to-stand, one leg stance, pull back against elastic resistance, external shoulder rotation against elastic resistance, and stepping up and down one step on stairs. For each exercise, 3 sets of 10 repetitions were performed, except for single leg stance, where 10 sets of 10 s were performed for each leg. The control exercise program consisted of five mobility exercises targeting hip, knee, ankle, trunk, chest, and shoulder mobility. All participants were instructed to perform the exercises three times per week.

Adherence to the vitamin D and omega-3s interventions was assessed by participant self-report at each 3-monthly contact (phone calls and clinical visits), by measuring 25(OH)D and polyunsaturated fatty acid blood levels and through capsule counts of used, partially used and full bottles of study capsules which were returned by the participants at each visit. Exercise adherence was assessed by participant self-report at each 3-monthly contact (phone calls and clinical visits). All participants were given a study diary to record their adherence to the study interventions and support reporting of adherence to the study staff at in-person and phone visits. The information in the diary was not used as a direct source of information for adherence.

Outcomes

Spine and proximal femur scans were acquired using dual-energy X-ray absorptiometry (DXA; Lunar iDXA, GE Healthcare machines were used at each of the four study sites) at each clinical visit (BL, 12, 24, and 36 months) and analyzed using enCORE software (Version 13.60.033). Predefined outcomes included in the DO-HEALTH study protocol included aBMD of the LS, FN, and TH. At baseline, both proximal femora were scanned, but only the side with the lower TH aBMD was measured at follow-up visits. If the side with lower aBMD at baseline became unsuitable for scanning, for example due to a fracture or endoprosthesis, the other side was used. For LS, L1-4 region was used and all evaluable vertebrae were included. If any of the vertebrae was affected by structure change or artifact, three or otherwise two vertebrae were included for analysis. If only one evaluable vertebra remained, the participant was excluded from data analysis. Criteria for exclusion of vertebrae included degenerative changes, fractures, artifacts (eg, aortic calcification), or difference in T-score to adjacent vertebra of ±1 SD.

TBS was measured as an additional, not predefined outcome, due its relevance to bone architecture and fracture risk. The integrated TBS software (TBS iNsight software v2.1; Medimaps, Geneva, Switzerland) was used for analysis. TBS analysis is based on standard spine DXA scans and quantifies variation in grey-level texture from 1 pixel to adjacent pixels. It provides an index of trabecular bone microstructure and is related to trabecular number, trabecular separation, and connectivity density.[30] Higher scores indicate stronger and fracture-resistant microarchitecture, whereas lower scores indicate deterioration in trabecular microarchitecture and higher risk for fracture. For TBS analyses, the same vertebrae were included as for LS aBMD. The TBS software is valid for use in individuals within the BMI range of 15–37 kg/m^2, and thus, individuals with BMI outside this range were excluded from TBS analyses.

Before the start of the trial, each study center performed 10 repeat scans of the European Spine Phantom which were used for cross-calibration of the four different DXA machines. Each of the DXA machines was calibrated daily throughout the trial. Participant positioning and scan acquisition were conducted according to standard operating procedures. A designated quality assurance center (Berlin) and experienced DXA technician (GA) ensured consistency in scan acquisition and analysis between the different densitometry sites. Other responsibilities included instrument quality control (monitoring of short- and long-term performance of DXA machines), and training and certification of all staff involved in DO-HEALTH DXA data collection.

The coefficient of variation (CV) for measurement reliability was established at the study center in Zurich, involving 3 DXA technicians and 30 participants. CV was 0.7% for LS, 1.2% for FN, and 0.9% for TH aBMD.

Statistical analyses and power calculations

Characteristics of study participants are described overall and by treatment group. Normally distributed continuous variables are presented as mean and standard deviation (SD) and non-normally distributed variables as median and interquartile range (IQR). Categorical variables are presented in frequencies and percentages.

The treatment effects on aBMD and TBS outcomes were analyzed based on the intent-to-treat principle. Separate mixed effects models with changes from baseline to 1, 2, and 3 years in aBMD and TBS as repeated outcomes were fit to data with robust standard errors. The mixed effects models were adjusted for the randomization stratification factors (study center, age, sex, prior fall, BMI, and the baseline level of the outcome). To determine whether the treatment effects are additive, three-way and two-way interactions were examined first. If none of the treatment interaction effects were significant ($P < .05$), three dichotomous indicators were added to the model for the main treatment effects. Otherwise, an eight-level treatment group categorical variable was added to the model. For each outcome, effects of treatment, time, and treatment by time interaction were examined using one regression model. Time was entered to

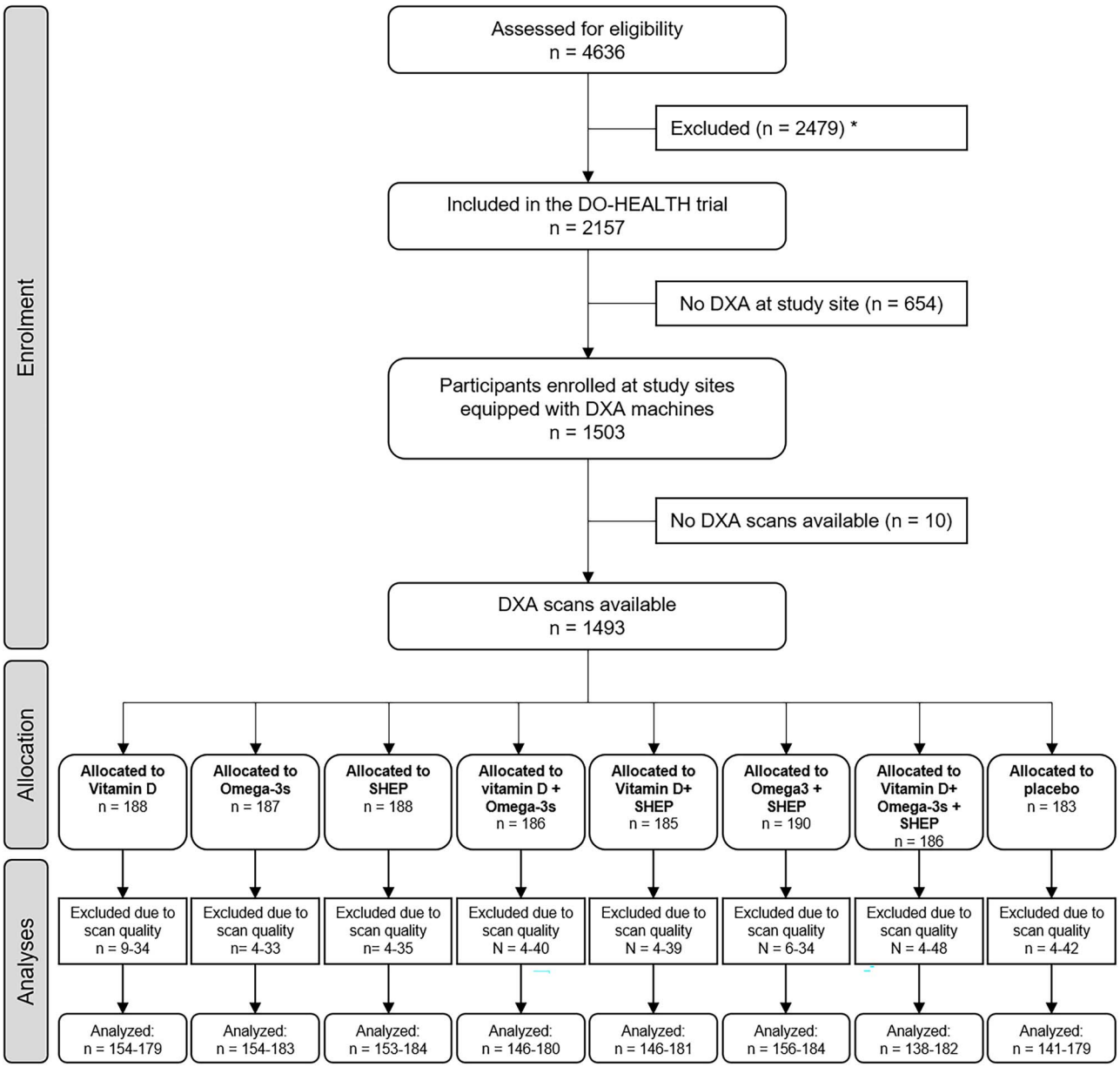

Figure 1. Flow of participants undergoing DXA scanning in the DO-HEALTH trial. *Reasons for exclusion are described in the DO-HEALTH primary outcome paper.[27]

the model as categorical variable. Adjusted means (AMs) and 95% confidence intervals are presented. The respective comparison group for additive effects of the treatments, that is when the dichotomous treatment variables are added to the model, is always the group that did not receive the respective samples. In case of synergistic treatment effects, each treatment combination is compared with placebo.

The following predefined subgroup analyses were performed: sex (female, male); age (70–74 vs. ≥75 years), baseline physical activity (below and above median of the Nurses' Health Study Physical Activity Questionnaire [NHS PAQ score]); dietary calcium intake (<1000 or ≥1000 mg), assessed by a comprehensive food frequency questionnaire; baseline serum 25(OH)D levels (<20 or ≥20 ng/mL); and fracture history in the 10 years prior to enrolment (yes/no), assessed by participant self-report. For subgroup analyses, significance of interactions between subgroup and treatment was assessed first. If the interaction was significant ($P < .05$), then stratified analyses were performed by each level of the subgroup factor. Significance level was set at 0.05 (two-sided) and analyses were performed in SAS v9.4 statistical software (Copyright© 2004 by SAS Institute Inc., Cary, NC, USA) or R Studio.

Power calculations were based on primary outcomes.[19] No a priori sample size calculations were conducted for BMD and TBS outcomes.

Results

Study participants

DXA scans were available for 1493 participants. The number of participants who underwent DXA scanning at follow up and could be included in the analyses was 1342 for FN aBMD, 1341 for TH aBMD, 1119 for LS aBMD, and 1098 for TBS. The CONSORT diagram of participant flow for this subsample of DO-HEALTH participants is shown in Figure 1. The mean age of participants was 74 years and 63% were female.

Baseline mean FN T-score was −1.4, and based on that score, 12% participants had osteoporosis, 55% had low bone mass (osteopenia), and 30% had healthy bone density at baseline. A history of low-trauma fractures in the 10 years prior to enrolment was reported by 162 (11%) participants and 7% took antiresorptive bone medications. Mean vitamin D levels at baseline were 21.9 ng/mL and 44% of participants were vitamin D deficient (25[OH]D < 20 ng/mL). Median dietary calcium intake (not including supplements) was 1297 mg/d, participants had good mobility (mean SPPB score at baseline 10.7) and were physically active (Nurses' Health Study questionnaire, mean METs: 36.6; 80.4% were moderately to vigorously physically active).

Baseline characteristics were generally balanced by treatment groups, except for LS and FN T-scores in the group randomized to omega-3s compared with those randomized to control (Table 1).

Adherence data to the study interventions in this study sample of 1496 DO-HEALTH participants with DXA measurements are presented in Supplementary Table S1. By year 3, ≥79% still took at least 80% of their total study pills and ≥65% performed the home exercise program at least twice per week.

Lumbar spine aBMD

For LS aBMD, there were no significant three- or two-way treatment interactions. Therefore, results from main effect analyses are presented and treatment effects are additive. For vitamin D, omega-3s, and SHEP, there were no significant differences between treatment and comparison groups across the 3 years of follow-up (Table 2). Also, there were no significant benefits for the combination of treatments over time (Supplementary Figure S1). However, there was a significant interaction for the subgroup by sex and vitamin D treatment ($P = .005$; Supplementary Table S2). Vitamin D versus no vitamin D had a beneficial effect on LS aBMD in males (ΔAMs: 0.0070 [95% CI, 0.0007, 0.0132] g/cm^2, $P = .029$) but not in females (Figure 2).

Femoral neck aBMD

For FN aBMD, there were no three- or two-way treatment interactions. Therefore, results from main effect analyses are presented and treatment effects are additive. For vitamin D, omega-3s, and SHEP, there were no significant differences between treatment and comparison groups across the 3 years (Table 2). Similarly, there were no significant effects of treatment combinations across the 3 years (Supplementary Figure S2). Regarding subgroup analyses, there were no significant interactions between any of the subgroups and treatments for FN aBMD (Supplementary Table S2).

Total hip aBMD

For TH aBMD, there were no three- or two-way treatment interactions. Therefore, results from main effect analyses are presented and treatment effects are additive. Vitamin D versus no vitamin D treatment had a significant positive effect on TH aBMD across the 3 years (ΔAMs: 0.0035 [95% CI, 0.0011, 0.0059] g/cm^2, $P = .005$). The positive effects of vitamin D were evident at year 2 (ΔAMs: 0.0042 [95% CI, 0.0014, 0.0070] g/cm^2) and year 3 (ΔAMs: 0.0045 [95% CI, 0.0012, 0.0078] g/cm^2; Table 2). Also the combination of vitamin D + omega-3s versus no vitamin D + no omega-3s showed a benefit across 3 years (ΔAMs: 0.0038 [95% CI, 0.0003, 0.0072] g/cm^2, $P = .033$; Figure 3), however only minimally different to vitamin D alone. Regarding subgroup analyses, there were no significant interactions between any of the subgroups and treatments for TH aBMD (Supplementary Table S2).

Lumbar spine TBS

For TBS, there was a significant three-way treatment interaction ($P = .012$); consequently, effects of the seven treatment combinations were compared with placebo. Here, only omega-3s plus SHEP compared with placebo had a significant positive effect on LS TBS across the 3 years (ΔAMs: 0.0115 [95% CI, 0.0011, 0.0219], $P = .030$; Supplementary Table S3). Regarding subgroup analyses, there were no significant interactions between any of the subgroups and treatments for TBS (Supplementary Table S4).

Discussion

In this 3-year, multi-center randomized controlled trial, including a subsample of 1493 DO-HEALTH participants with DXA measurements at baseline, 12, 24, and 36 months follow-up, supplementation with 2000 IU vitamin D3 per day had a small benefit on TH aBMD in all participants, and a small benefit on LS aBMD in men. However, there was no benefit of vitamin D on other aBMD sites. Also, the simple home exercise program and omega-3s treatment showed no effects on aBMD in generally healthy, largely vitamin D replete and active adults age 70 and older. In an exploratory analysis of TBS, there was a suggestion that the combination of SHEP and 1 g of marine omega-3s per day may improve TBS. Given the small effect sizes, the clinical impact of our findings is unclear.

The observed small benefit of vitamin D treatment on TH aBMD in DO-HEALTH (3-year change: 0.0035 g/cm^2, ≈0.39%) is consistent with a large meta-analysis of 41 clinical trials (0.34%, 95% CI, 0.13–0.55)[31,32] but was not confirmed in an ancillary study of the VITAL trial among 771 older adults (mean age 64 years).[33] The difference in findings between VITAL and DO-HEALTH for the same daily dose of 2000 IU vitamin D may be explained by the larger sample size in DO-HEALTH for this outcome, the older age group enrolled in DO-HEALTH (mean age 74 years in DO-HEALTH vs. 64 years in VITAL) and possibly by the lower baseline 25(OH)D levels in DO-HEALTH participants (21.9 ng/mL in DO-HEALTH vs. 27.7 ng/mL). Additionally, we found a beneficial effect of vitamin D on spine aBMD, although it was limited to men. This sex-specific benefit on spine aBMD remains unclear, but may possibly be attributed a greater proportion of males with vitamin D deficiency at baseline (male: 48.7% vs. female: 40.9%) and a somewhat lower baseline calcium intake in males (1253 vs. 1326 mg/d). Consideration of multiple comparisons is warranted when interpreting these findings. Furthermore, effects on both, TH and LS aBMD, are considerably smaller than the suggested aBMD difference required to show a fracture risk reduction (1.4% for vertebral and 3.2% for hip fractures),[34] and thus, clinical impact of our findings may be limited.

To the best of our knowledge, this is the largest RCT reporting the effect of supplemental omega-3s on aBMD in older adults. Our findings align with a meta-analysis that compared higher (aim for ≥10% increase in total intake, from

Table 1. Baseline characteristics of study participants.

Characteristics	Overall ($n = 1493$)	Vitamin D		P	Omega-3 s		P	Exercise		P
		Vitamin D ($n = 745$)	No vitamin D ($n = 748$)		Omega-3 s ($n = 749$)	No omega-3 s ($n = 744$)		SHEP ($n = 749$)	Control exercise ($n = 744$)	
Age [yrs], median [IQR][a]	74.0 [71.0, 77.0]	74.0 [71.0, 78.0]	74.0 [72.0, 77.0]	0.786	74.00 [71.00, 77.00]	74.00 [72.00, 77.25]	0.493	74.00 [71.00, 77.00]	74.00 [71.75, 77.00]	0.756
BMI [kg/m^2], mean (SD)[b]	26.63 (4.31)	26.75 (4.42)	26.50 (4.19)	0.270	26.61 (4.25)	26.64 (4.37)	0.919	26.64 (4.29)	26.61 (4.34)	0.887
Female, *n* (%)	945 (63.3)	477 (64.0)	468 (62.6)	0.595	476 (63.6)	469 (63.0)	0.879	473 (63.2)	472 (63.4)	0.950
Comorbidity score, median [IQR][a, c]	3.0 [1.0, 5.0]	3.0 [1.0, 5.0]	3.0 [1.0, 5.0]	0.983	3.00 [1.00, 5.00]	3.00 [1.00, 5.00]	0.992	2.00 [1.00, 5.00]	3.00 [1.00, 5.00]	0.253
LS T-score, mean (SD)	−1.27 (1.43)	−1.29 (1.37)	−1.26 (1.48)	0.806	−1.41 (1.35)	−1.14 (1.50)	0.001	−1.25 (1.38)	−1.30 (1.48)	0.562
FN T-score, mean (SD)	−1.43 (1.00)	−1.44 (0.96)	−1.43 (1.03)	0.812	−1.49 (0.97)	−1.37 (1.03)	0.018	−1.42 (0.99)	−1.44 (1.01)	0.747
Bone status based on FN T-score[d]				0.067			0.261			0.357
Healthy, *n* (%)	451 (30.3)	213 (28.7)	238 (32.0)		219 (29.4)	232 (31.3)		224 (30.0)	227 (30.7)	
Osteopenia, *n* (%)	814 (54.8)	427 (57.5)	387 (52.0)		403 (54.2)	411 (55.4)		423 (56.6)	391 (52.9)	
Osteoporosis, *n* (%)	181 (12.2)	79 (10.6)	102 (13.7)		103 (13.8)	78 (10.5)		82 (11.2)	99 (13.8)	
Bone medication intake, *n* (%)	109 (7.3)	56 (7.5)	53 (7.1)	0.825	57 (7.6)	52 (7.0)	0.718	54 (7.2)	55 (7.4)	0.971
Fracture history, *n* (%)	162 (10.9)	87 (11.7)	75 (10.0)	0.325	73 (9.7)	89 (12.0)	0.106	75 (10.0)	87 (11.7)	0.484
SPPB score, mean (SD)[e]	10.72 (1.55)	10.76 (1.53)	10.68 (1.56)	0.335	10.66 (1.62)	10.77 (1.47)	0.197	10.71 (1.55)	10.72 (1.55)	0.846
Daily calcium intake [mg], median [IQR][a,f]	1297 [1006, 1724]	1284 [1010, 1694]	1301 [1005, 1771]	0.423	1292 [1017, 1730]	1302 [1005, 1720]	0.921	1322 [1031, 1724]	1285 [981, 1725]	0.278
Physical activity level, *n*(%)				0.05			0.927			0.927
Inactive	293 (19.6)	128 (17.1)	165 (22.1)		149 (20.0)	144 (19.3)		149 (20.0)	144 (19.3)	
Moderately active (1–3 times/week)	474 (31.8)	247 (33.1)	227 (30.5)		236 (31.7)	238 (31.8)		236 (31.7)	238 (31.8)	
Active (>3 times/week)	725 (48.6)	372 (49.8)	353 (47.4)		359 (48.3)	366 (48.9)		359 (48.3)	366 (48.9)	
Serum 25-hydroxyvitamin D concentration [ng/mL], mean (SD)	21.85 (8.36)	21.95 (8.44)	21.75 (8.28)	0.633	21.76 (8.37)	21.94 (8.36)	0.682	22.33 (8.50)	21.37 (8.19)	0.028
Vitamin D deficiency (<20 ng/mL), *n* (%)	648 (43.8)	311 (42.1)	337 (45.4)	0.232	323 (43.5)	325 (44.0)	0.904	312 (42.1)	336 (45.4)	0.220

Abbreviations: BMI, body mass index; FN, femoral neck; IQR, interquartile range; LS, lumbar spine; SHEP, strength-training home exercise program; SPPB, short physical performance battery; yrs, years. [a]Median and IQR are presented for non-normally distributed variables [b]BMI was calculated as weight in kilograms divided by height in meters squared. [c]Comorbidity was measured by the Self-Administered Comorbidity Questionnaire, which assesses 12 comorbidities by 3 dimensions (presence, medication, and limitation of activities). It has a range of 0–36 points and lower scores indicate better health. [d]A T-score of >1.0 was defined as healthy, ≤−1.0 to >−2.5 as osteopenia and ≤−2.5 as osteoporosis. [e]The SPPB assesses lower extremity function. Scores range from 0 to 12, in which higher scores are better. [f]Assessed by an electronic food frequency questionnaire, specifically developed for DO-HEALTH and targeted to older adults.

Table 2. Change from baseline in aBMD outcomes [g/cm^2].

	Vitamin D	No vitamin D	Difference (95% CI)	*P*-value	Omega-3 s	No Omega-3 s	Difference (95% CI)	*P*-value	SHEP	Control exercise	Differences (95% CI)	*P*-value
LS **aBMD**												
Unadjusted at baseline,	1.0398	1.0440	−0.0043	0.692	1.0262	1.0577	−0.0315	0.003	1.0414	1.0367	0.0085	0.428
n = 1214	(1.0268, 1.0567)	(1.0292, 1.0589)	(−0.0253, 0.0168)		(1.0113, 1.0411)	(1.0428, 1.0725)	(−0.0525, −0.0104)		(1.0264, 1.0565)	(1.0217, 1.0517)	(−0.0125, 0.0296)	
Adjusted change from baseline												
Year 1	0.0027	0.0020	0.0008		0.0021	0.0025	−0.0004		0.0004	0.0043	−0.0040	
	(−0.0, 0.0054)	(−0.0007, 0.0046)	(−0.0030, 0.0045)		(0.0006, 0.0048)	(−0.0002, 0.0052)	(−0.0042, 0.0034)		(−0.0024, 0.0031)	(0.0016, 0.0070)	(−0.0077, −0.0002)	
Year 2	0.0028	0.0032	−0.0004		0.0023	0.0036	−0.0013		0.0033	0.0026	0.0007	
	(−0.0004, 0.0059)	(0.0, 0.0063)	(−0.0048, 0.0040)		(−0.0009, 0.0055)	(0.0005, 0.0068)	(−0.0058, 0.0031)		(0.0002, 0.0065)	(−0.0005, 0.0057)	(−0.0037, 0.0051)	
Year 3	0.0044	0.0032	0.0011		0.0032	0.0044	−0.0011		0.0034	0.0042	−0.0008	
	(0.0008, 0.0080)	(−0.0003, 0.0068)	(0.0039, 0.0062)		(−0.0004, 0.0068)	(0.0008, 0.0079)	(−0.0062, 0.0039)		(−0.0002, 0.0070)	(0.0007, 0.0078)	(−0.0059, 0.0042)	
Average across 3 years	0.0033	0.0028	0.0005	0.789	0.0026	0.0035	−0.0010	0.614	0.0024	0.0037	−0.0014	0.477
	(0.0006, 0.0060)	(0.0001, 0.0054)	(−0.0032, 0.0042)		(−0.0001, 0.0052)	(0.0008, 0.0062)	(−0.0047, 0.0028)		(−0.0003, 0.0050)	(0.0011, 0.0064)	(−0.0051, 0.0024)	
FN **aBMD**												
Unadjusted at baseline,	0.8343	0.8375	−0.0032	0.639	0.8280	0.8438	−0.0158	0.022	0.8373	0.8345	0.0028	0.681
n = 1452	(0.8247, 0.8439)	(0.8280, 0.8471)	(−0.0168, 0.01030)		(0.8184, 0.8376)	(0.8342, 0.8534)	(−0.0294, −0.0023)		(0.8278, 0.8469)	(0.8249, 0.8441)	(−0.0107, 0.0164)	
Adjusted change from baseline												
Year 1	0.0071	0.0077	−0.0006		0.0053	0.0095	−0.0043		0.0069	0.0080	−0.0011	
	(0.0040, 0.0102)	(0.0047, 0.0108)	(−0.0049, 0.0036)		(0.0022, 0.0093)	(0.0065, 0.0126)	(−0.0085, 0)		(0.0038, 0.0099)	(0.0049, 0.0110)	(−0.0054, 0.0032)	
Year 2	0.0030	0.0037	−0.0007		0.0012	0.0054	−0.0042		0.0023	0.0043	−0.0020	
	(−0.0003, 0.0062)	(0.0005, 0.0069)	(−0.0052, 0.0037)		(−0.0020, 0.0044)	(0.0022, 0.0086)	(−0.0087, 0.0003)		(−0.0009, 0.0056)	(0.0011, 0.0075)	(−0.0064, 0.0025)	
Year 3	−0.0017	−0.0047	0.0030		−0.0046	−0.0018	−0.0027		−0.0040	−0.0024	−0.0016	
	(−0.0054, 0.0020)	(−0.0083, −0.0010)	(−0.0022, 0.0081)		(−0.0082, −0.0009)	(−0.0055, 0.0018)	(−0.0079, 0.0024)		(−0.0077, −0.0003)	(−0.0061, 0.0013)	(−0.0067, 0.0035)	
Average across 3 years	0.0028	0.0022	0.0005	0.800	0.0006	0.0044	−0.0037	0.076	0.0017	0.0033	−0.0016	0.461
	(−0.0002, 0.0058)	(−0.0007, 0.0052)	(−0.0036, 0.0047)		(−0.0023, 0.0036)	(0.0014, 0.0073)	(−0.0079, 0.0004)		(−0.0012, 0.0047)	(0.0003, 0.0062)	(−0.0057, 0.0026)	
TH **aBMD**												
Unadjusted at baseline,	0.8981	0.8983	−0.0002	0.979	0.8899	0.9066	−0.0167	0.029	0.9000	0.8967	0.0033	0.663
n = 1451	(0.8875, 0.9088)	(0.8877, 0.9089)	(−0.0152, 0.0148)		(0.8793, 0.9005)	(0.8960, 0.9172)	(−0.0317, −0.0017)		(0.8893, 0.9105)	(0.8859, 0.9072)	(−0.0117, 0.0184)	
Adjusted change from baseline												
Year 1	0.0022	0.0003	0.0019		0.0013	0.0012	0.0001		0.0005	0.0020	−0.0016	
	(0.0005, 0.0039)	(−0.0014, 0.0020)	(−0.0005, 0.0043)		(−0.0004, 0.0030)	(−0.0006, 0.0029)	(−0.0023, 0.0025)		(−0.0013, 0.0022)	(0.0003, 0.0037)	(−0.0040, 0.0008)	
Year 2	−0.0009	−0.0051	0.0042		−0.0031	−0.0028	−0.0003		−0.0039	−0.0020	−0.0019	
	(−0.0029, 0.0011)	(−0.0071, −0.0031)	(0.0014, 0.0070)		(−0.0051, −0.0011)	(−0.0048, −0.0008)	(−0.0031, 0.0025)		(−0.0059, −0.0019)	(−0.0040, −0.0000)	(−0.0047, 0.0009)	
Year 3	−0.0053	−0.0098	0.0045		−0.0071	−0.0080	0.0009		−0.0085	−0.0066	−0.0019	
	(−0.0077, −0.0030)	(−0.0121, −0.0075)	(0.0012, 0.0078)		(−0.0095, −0.0048)	(−0.0104, −0.0057)	(−0.0024, 0.0042)		(−0.0109, −0.0062)	(−0.0090, −0.0043)	(−0.0052, 0.0014)	
Average across 3 years	−0.0013	−0.0049	0.0035	0.005	−0.0030	−0.0032	0.0002	0.840	−0.0040	−0.0022	−0.0018	0.147
	(−0.0031, 0.0004)	(−0.0066, −0.0031)	(0.0011, 0.0059)		(−0.0047, −0.0012)	(−0.0050, −0.0015)	(−0.0022, 0.0027)		(−0.0057, −0.0023)	(−0.0039, −0.0005)	(−0.0042, 0.0006)	

Abbreviations: aBMD, areal bone mineral density; SHEP, strength-training home exercise program. Analyses were adjusted for age, sex, BMI, prior fall, study site, and baseline level of the outcome.

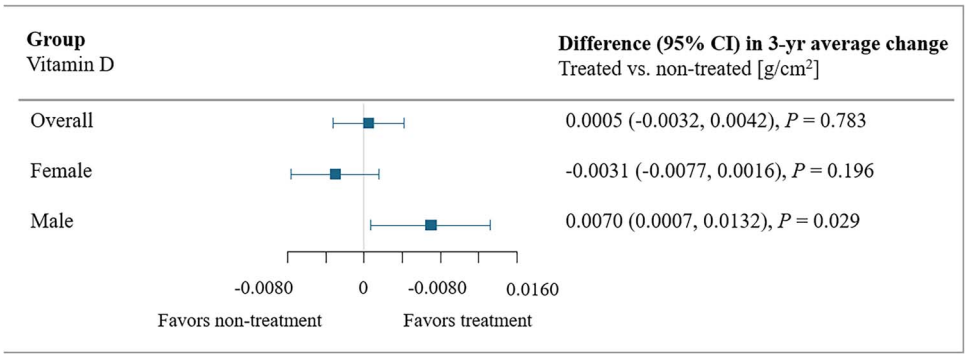

Figure 2. 3-year average change in LS aBMD for vitamin D versus no vitamin D overall and by sex. Abbreviations: aBMD, areal bone mineral density; CI, confidence interval; yr, year. Analyses were adjusted for age, sex, BMI, prior fall, study site, and baseline LS aBMD, $N = 1119$.

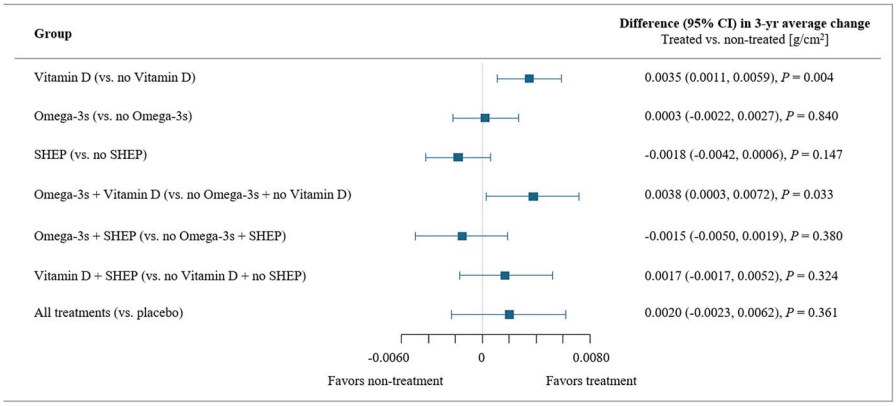

Figure 3. 3-year average change in TH aBMD for treatment combinations compared with the respective group not receiving the treatment. Abbreviations: aBMD, areal bone mineral density; CI, confidence interval; SHEP, strength-training home exercise program; yr, year. Analyses were adjusted for age, sex, BMI, prior fall, study site, and baseline TH aBMD. $N = 1341$.

diet or supplements) versus lower omega-3s intake and concluded no benefit at any of the measuring sites.[22] In contrast, a recent meta-analysis of RCTs among middle-aged adults (≥50 years) reported a small benefit of any type of omega-3s supplementation (0.005 g/cm^2, 95% CI, 0.000–0.010; 7 interventions), most pronounced for EPA and DHA combined (0.026 g/cm^2, 95% CI, 0.022–0.030).[23] However, that meta-analysis is restricted by the small number of included trials, small sample sizes per trial (<100 participants/group), and lack of specification of which bone site (eg, LS, FN) was used. Based on our findings and existing evidence, there is no high-quality evidence to support omega-3s supplementation to improve bone health in generally healthy older adults today.

The beneficial effects of exercise on aBMD and bone microarchitecture have been well established.[26,35] There are two main reasons that may explain the lack of benefit of the DO-HEALTH exercise program on aBMD. First, the SHEP program was of relatively low intensity and included minimal external resistance (ie, light elastic band) which also was not progressed in intensity across the 3 years. Second, 80% of DO-HEALTH participants were already engaging in moderate to vigorous physical activity at baseline.[27] As suggested by the literature, in order to have an osteogenic effect, an exercise intervention needs to exceed habitual bone loading.[36] Therefore, our exercise program in the generally healthy and active DO-HEALTH population may have been insufficient to stimulate an effect on aBMD.[36] It is important to note that our findings do neither invalidate the benefits of exercise for bone health and healthy aging, nor do they invalidate previous beneficial effects of SHEP for fall and fracture prevention in frail older adults with acute hip fracture.[37]

As an exploratory outcome, we also assessed TBS, which is still a relatively novel analytical tool in the evaluation of trabecular microstructure, osteoporosis, and fracture risk.[30,38] Few trials to date have used TBS to evaluate effects of vitamin D,[38–41] omega-3 s,[43] or exercise,[44] and the majority of those trials are limited by small sample sizes (eg, ≤200 participants)[39,40,41–43] and short follow-up durations[45] (≤6 months).[39,40,44] DO-HEALTH was the first trial to examine individual and combined effects of vitamin D, omega-3s, and exercise on TBS and suggests a synergistic effect of combined omega-3s supplementation and SHEP across the 3-year follow-up. Of note, this effect only became significant at 3 years.

This study has several limitations. First, study participants were not selected for vitamin D deficiency, low bone mass, or increased fracture risk at baseline, which may have limited the ability to detect a significant treatment effect. Second, there were some imbalances in baseline LS and FN T-score for the omega-3 s versus no omega-3s groups. This limitation was addressed by adjusting all analyses for baseline level of the outcome measure. Third, the observed effects of 0.0035 gcm^2 (≈0.4%) at the TH and 0.0070 g/cm^2 (≈0.7%) at the LS across the 3 years are relatively small and did not exceed LSC measured at the study center in Zurich (TH: 0.022 g/cm^2, spine: 0.021 g/cm^2).[46] Fourth, we did not adjust the P values

for multiple testing. Given the large number of comparisons, the significance threshold of $P = .05$ may have been too liberal, and statistically significant results may have occurred by chance. Finally, restriction of recruitment to generally healthy older adults, unselected for low bone mass or osteoporosis at baseline, limits generalizability of our findings to populations at risk for osteoporosis and fractures but also defines a conservative approach for the three interventions tested.

In conclusion, we observed a benefit of 2000 IU of supplemental vitamin D3 per day on total hip aBMD overall and on lumbar spine aBMD in males; however, effects were small and clinical signficiance of the findings thus unclear. Omega-3s supplementation and a simple strength training home exercise program appear to have no benefits for aBMD in generally healthy older adults.

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Supplementary material

Supplementary material is available at *Journal of Bone and Mineral Research* online.

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Conflicts of interest

G.A. declares institutional grants from Bayer AG, Madrigal Pharmaceuticals, and Deutsches Zentrum für Luft- und Raumfahrt (DLR), consulting fees from Porous GmbH and honoraria from Landesanstalt für Personendosimetrie und Strahlenschutzausbildung. J.A.K. is a board member of IOF, ESCEO, and NOGG, and a director of Osteoporosis Research Ltd. (UK) and Patron of Osteoporosis 2000 (UK). L.C.H. declares consulting fees from Amgen, Ascendis, UCB, Pharmacosmos and honoraria from NovoNordisk. R.C.S. declares an employee contract and stock with Roche, honoraria for lectures and testimony by the Hirslanden hospital group, and Innosuisse, respectively, and participation in an advisory board with Hygiaso. J.A.P.D. has received speaker fees from AMGEN and was the scientific director of FORUM D, an industry-sponsored online platform in Portuguese dedicated to the dissemination of evidence regarding Vitamin D effects on health. H.A.B.-F. reports as the PI of the DO-HEALTH trial, grants from the European Commission (Grant Agreement No. 278588), from the University of Zurich, from NESTEC, from PFIZER Consumer Healthcare, from Streuli Pharma, plus non-financial support from DSM Nutritional Products and from Roche Diagnostics. Furthermore, H.A.B.-F. reports speaker fees from Wild, Pfizer, Vifor, Mylan, Roche Diagnostics, and independent and investigator initiated grants from Pfizer and from Vifor, outside the submitted work. M.K.-F., S.G., R.T., R.R., B.D.-H., B.V., E.J.O., R.W.K., A.E., W.L., G.A.W. declare no conflict of interest.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

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