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Original Research

Effects of Vitamin D, Omega-3 Fatty Acids and a Home Exercise Program on Prevention of Pre-Frailty in Older Adults: The DO-HEALTH Randomized Clinical Trial

M. Gagesch[1,2], M. Wieczorek[1,2], B. Vellas[3], R.W. Kressig[4], R. Rizzoli[5], J. Kanis[6,7], W.C. Willett[8,9], A. Egli[2], W. Lang[1,2], E.J. Orav[10], H.A. Bischoff-Ferrari[1,2,11]

1. Department of Aging Medicine, University Hospital Zurich, Zurich, Switzerland; 2. Centre on Aging and Mobility, University Hospital Zurich and University of Zurich, Zurich, Switzerland; 3. Gérontopôle, Toulouse University Hospital, University of Toulouse, UMR INSERM 1295, Toulouse, France; 4. University Department of Geriatric Medicine FELIX PLATTER, Basel, Switzerland; 5. Service of Bone Diseases, Geneva University Hospitals and Faculty of Medicine, Geneva, Switzerland; 6. Mary McKillop Institute for Health Research, Australian Catholic University, Melbourne, Australia; 7. Centre for Metabolic Bone Diseases, University of Sheffield Medical School, Sheffield, UK; 8. Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, USA; 9. Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, USA; 10. Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, USA; 11. University Clinic for Aging Medicine, City Hospital Zurich, Zurich, Switzerland; ClinicalTrials.gov Identifier: NCT01745263

Corresponding Author: Michael Gagesch, MD, Department of Aging Medicine, University Hospital Zurich, Rämistrasse 100, 8091 Zurich, Switzerland, Email: michael.gagesch@usz.ch

Abstract

BACKGROUND: The benefits of supplemental vitamin D3, marine omega-3 fatty acids, and a simple home exercise program (SHEP) on frailty prevention in generally healthy community-dwelling older adults are unclear.

OBJECTIVE: To test the effect of vitamin D3, omega-3s, and a SHEP, alone or in combination on incident pre-frailty and frailty in robust older adults over a follow-up of 36 months.

METHODS: DO-HEALTH is a multi-center, double-blind, placebo-controlled, 2x2x2 factorial randomized clinical trial among generally healthy European adults aged 70 years or older, who had no major health events in the 5 years prior to enrollment, sufficient mobility and intact cognitive function. As a secondary outcome of the DO-HEALTH trial, among the subset of participants who were robust at baseline, we tested the individual and combined benefits of supplemental 2,000 IU/day of vitamin D3, 1 g/day of marine omega-3s, and a SHEP on the odds of being pre-frail and frail over 3 years of follow-up.

RESULTS: At baseline, 1,137 out of 2,157 participants were robust (mean age 74.3 years, 56.5% women, mean gait speed 1.18 m/s). Over a median follow-up time of 2.9 years, 696 (61.2%) became pre-frail and 29 (2.6%) frail. Odds ratios for becoming pre-frail were not significantly lower for vitamin D3, or omega 3-s, or SHEP, individually, compared to control (placebo for the supplements and control exercise). However, the three treatments combined showed significantly decreased odds (OR 0.61 [95% CI 0.38-0.98; p=0.04) of becoming pre-frail compared to control. None of the individual treatments or their combination significantly reduced the odds of becoming frail.

CONCLUSION: Robust, generally healthy and active older adults without major comorbidities, may benefit from a combination of high-dose, supplemental vitamin D3, marine omega-3s, and SHEP with regard to the risk of becoming pre-frail over 3 years.

Key words: Frailty prevention, clinical trial, older adults.

Introduction

Physical frailty is an age-related medical syndrome affecting the health of older adults, leading to an increased susceptibility of the individual against multiple negative health outcomes (1, 2). With the ongoing growth of the global aging population, the impact of frailty on the individual and also on the health economic level is expected to increase substantially (3). Of note, frailty is a dynamic process and is potentially reversible if detected early (4, 5). While existing evidence suggests a noteworthy role for vitamin D, omega-3s and physical activity on the progression of frailty, the combination of low-cost interventions including the supplementation of key nutrients and increased exercise might appear favorable also for the primary prevention of frailty (6, 7).

Low 25-hydroxyvitamin D (Calcidiol) levels have been associated with frailty (8, 9) and pre-frailty (10) in several observational studies of community-dwelling adults. Mechanistically, it has been suggested that vitamin D supplementation may reduce frailty via several pathways, including positive effects on muscle and bone health (11, 12). Consequently, Vitamin D supplementation might play an important role in the prevention of frailty and the at-risk state of pre-frailty. However, evidence from the current literature is scarce and results from randomized controlled trials are still missing (13, 14).

Omega 3-fatty acids have been linked to skeletal muscle health (6). Mechanistically, it has been suggested that omega-3s reduce pro-inflammatory cytokines, improve insulin sensitivity, stimulate muscle protein synthesis via the mTOR signaling pathway, and reduce reactive oxygen species (ROS) in mitochondria (6, 15-18). Although evidence so far appears limited, omega-3 supplementation may provide relevant benefits on muscle function in older adults (19).

Low physical activity is a key component of physical frailty

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(20). Mechanistically, low physical activity has been associated with inflammaging and impaired mitochondrial function leading to reduced oxidative capacity linked to the development of sarcopenia and frailty (21-23).

Prior results from observational studies suggested that slowness, low activity level and weakness separate frail and non-frail groups already 6 years prior to clinically overt onset of frailty (RR ranging from 1.39 - 1.94) (24). These findings indicate the importance of low physical performance on the trajectory towards frailty. Further, the combination of supplemental vitamin D and physical exercise might provide an additive effect on muscle function in older adults (25).

In regard to health and active aging, preventing pre-frailty among robust older adults appears important as pre-frail individuals are already at risk of adverse outcomes including falls, disability, hospitalizations and premature mortality (2). The DO-HEALTH study of generally healthy older European adults reported that 43% of their participants at baseline were considered pre-frail (26). Therefore, it is critical to timely identify potential interventions to address pre-frailty for the prevention of frailty and its associated negative health outcomes in older adults (27).

Randomized trials assessing the treatment of vitamin D3, omega-3s supplementation, and home exercise, alone or in combination, for the primary prevention of pre-frailty and frailty are missing to date (28). Therefore, we aim to test the individual and combined effects of treatment with vitamin D3, omega-3s, and a simple home exercise program (SHEP) for the prevention of pre-frailty and frailty in generally healthy and robust adults age 70 and older from the large DO-HEALTH trial.

Methods

Trial design and oversight

DO-HEALTH is a three–year, multi-center, double-blind, 2x2x2 factorial design randomized controlled clinical trial (NCT01745263) designed to support healthy aging in European adults age 70 and older (29, 30). Clinical visits were at baseline, 1, 2, and 3 years, with intermediate phone calls every three months. The detailed trial design and protocol have been described elsewhere (29, 30). The 2,157 trial participants were generally healthy, community-dwelling adults aged 70 years and older, recruited from seven centers in five European countries: Zurich, Basel, Geneva (Switzerland); Berlin (Germany); Innsbruck (Austria); Toulouse (France); and Coimbra (Portugal). Inclusion criteria were absence of major health events in the five years prior to enrolment including cancer and cardiovascular events, sufficient mobility to come to the study centers, and intact cognitive function with a MMSE score of at least 24 points. All participants signed informed consent and the study protocol was approved by ethical and regulatory agencies of all five countries.

Interventions

The three interventions were randomly assigned in eight treatment groups according to the 2x2x2 factorial trial design: 2000 IU/d of vitamin D3,1 g/d of omega-3s and SHEP (n = 264); vitamin D3 and marine omega-3s (n = 265); vitamin D3 and SHEP (n = 275); vitamin D3 alone (n = 272); omega-3s and SHEP (n = 275); omega-3s alone (n = 269); SHEP alone (n = 267); or placebo (n = 270). Each participant received two study capsules per day: each active vitamin D capsule contained 1000 IU of Vitamin D3 stabilized with dl-α-tocopherol (vitamin E, 2.5 pro mill); each active omega-3s capsule contained 500 mg of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in a ratio of 1:2; and each placebo capsule contained high oleic sunflower oil. The SHEP was an unsupervised strength-training exercise of 30 minutes, three times per week compared with an attention control exercise program focused on joint flexibility of 30 minutes three times a week (details of the program are presented in Supplementary Table 1). All participants were required to limit the use of vitamin D from all other supplemental sources, including multivitamins, to the recommended dietary allowance intake for older adults (800 IU per day), and to forego any supplemental omega-3 intake.30 In addition, participants were provided with a personal diary to record their adherence to the study interventions and encouraged to implement an exercise routine in order to allow optimal adherence to the SHEP. Study personnel collected the diary and documented the routine at each clinical visit. Details on adverse events and adherence have been reported earlier (29).

Operationalization of pre-frailty and frailty

Frailty status was assessed at baseline and annually over three years of follow-up according to the five domains of the Fried physical frailty phenotype (20), with limited adaptions to the five domains to fit the variables available from the DO-HEALTH dataset. For weakness, grip strength was recorded in Kilopascal (kPa) from the best of three consecutive trials using the dominant hand with a Martin Vigorimeter (KLS Martin Group, Tuttlingen, Germany). We used cut points determined by the lowest quintile approach as did Fried and colleagues in their landmark study, stratified by sex and age group (<75 years and ≥75 years) (31). Fatigue was defined by a positive answer to the self-reported question: “In the last month, have you had too little energy to do things you wanted to?” from the Survey of Health, Ageing and Retirement in Europe (SHARE) Frailty Instrument (SHARE-FI) (32). Involuntary relevant weight loss was defined as >4.5kg or a >5% change in weight in 1 year at follow-up. Low gait speed was defined as ≤0.65m/s (men ≤173cm, women ≤159cm) and ≤0.76 (men >173cm, women >159cm) from the Short Physical Performance Battery (SPPB) (33). Finally, low activity level was defined as a response of “Less than once a week» to the self-report question: “How often do you engage in activities that require a low or moderate level of energy such as gardening, cleaning the car, or going on a walk?” from SHARE-FI (32). Participants with

Table 1. Baseline characteristics of the DO-HEALTH trial participants that were robust at baseline and received individual treatments (n=1,137)

	Vitamin D			Omega-3			Exercise			Overall
	Vitamin D	No Vitamin D		Omega-3	No Omega-3		SHEP	Control exercise		Total
	N=562	N=575	P	N=563	N=574	P	N=581	N=556	P	N=1,137
Age, years, mean (SD)	74.5 (4.1)	74.2 (3.9)	0.272	74.2 (3.8)	74.5 (4.2)	0.1813	74.5 (4.1)	74.1 (3.9)	0.071	74.3 (4.0)
BMI, kg/m2, mean (SD)	26.2 (4.1)	26.0 (3.9)	0.2286	26.1 (4.1)	26.1 (4.0)	0.7786	26.3 (4.0)	25.9 (4.1)	0.1883	26.1 (4.0)
Sex, No. (%)			0.9361			0.184			0.1858	
Women, n (%)	318 (56.6)	324 (56.4)		329 (58.4)	313 (54.5)		317 (54.5)	325 (58.5)		642 (56.5)
Men, n (%)	244 (43.4)	251(43.7)		234 (41.6)	261 (45.4)		264 (45.4)	231 (41.6)		495 (43.5)
Living alone, n (%)	216 (38.4)	216 (37.6)	0.7628	226 (40.1)	206 (35.9)	0.1396	209 (36.0)	223 (40.1)	0.151	432 (38.0)
Education, years, mean (SD)	13.2 (4.1)	13.2 (3.8)	0.8831	13.3 (3.7)	13.2 (4.1)	0.6393	13. (3.9)	13.3 (3.9)	0.4399	13.2 (3.9)
Comorbidity score*, mean (SD)	1.4 (1.2)	1.4 (1.2)	0.7571	1.4 (1.2)	1.4 (1.2)	0.6819	1.4 (1.2)	1.4 (1.2)	0.3095	1.4 (1.2)
Number of medications, mean	2.7 (2.4)	2.7 (2.5)	0.9287	2.6 (2.4)	2.8 (2.4)	0.3144	2.7 (2.5)	2.7 (2.3)	0.7123	2.7 (2.4)
SPPB score, median (IQR)	11.3 (1.1)	11.2 (1.1)	0.2406	11.3 (1.1)	11.3 (1.1)	0.8541	11.2 (1.1)	11.3 (1.1)	0.352	11.3 (1.1)
Gait speed m/s, mean (SD)	1.18 (0.20)	1.18 (0.21)	0.9795	1.18 (0.20)	1.17 (0.21)	0.4055	1.17 (0.20)	1.18 (0.21)	0.4372	1.18 (0.20)
Use of walking aids, n (%)	5 (0.9)	6 (1.1)	0.8003	6 (1.1)	5 (0.9)	0.7329	10 (1.7)	1 (0.2)	0.0077	11 (1.0)
Prior fall, n (%)	224 (39.9)	215 (37.4)	0.3931	227 (40.3)	212 (36.9)	0.241	225 (38.7)	214 (38.5)	0.9346	439 (38.6)
Vitamin D supplement users (≤ 800 IU), n (%)	51 (9.1%)	61 (10.6%)	0.3855	64 (57.1%)	48 (8.4%)	0.0891	56 (9.6%)	56 (10.1%)	0.8063	112 (9.9)
Vitamin D# deficiency (≥12, <20 ng/mL), n (%)	156 (13.8)	165 (14.6)	0.9326	155 (13.6)	166 (14.7)	0.6765	164 (14.4)	157 (13.9)	0.7555	321 (28.4)
Severe Vitamin D# deficiency (<12 ng/mL), n (%)	56 (4.9)	55 (4.9)	0.9326	59 (5.2)	52 (4.6)	0.6765	53 (4.7)	58 (5.1)	0.7555	111 (9.8)
Serum 25-hydroxyvitamin D concentration, ng/mL, mean (SD)	23.0 (8.3)	22.9 (8.4)	0.9182	22.9 (8.4)	23.0 (8.3)	0.9441	23.2 (8.7)	22.7 (8.0)	0.2597	23.0 (8.3)
Serum DHA concentration, μg/mL, mean (SD)	75.6 (33.1)	76.1 (33.7)	0.7915	76.6 (34.4)	75.1 (32.2)	0.4505	75.9 (33.2)	75.7 (33.6)	0.9033	75.8 (33.4)
Serum EPA concentration, μg/mL, mean (SD)	28.7 (16.5)	30.4 (17.6)	0.0917	29.6 (16.8)	29.5 (17.5)	0.8886	29.3 (17.2)	29.7 (17.1)	0.6829	29.5 (17.1)
MET hours/week, mean (SD)	38.9 (32.0)	41.7 (33.7)	0.1496	40.8 (33.1)	39.9 (32.7)	0.6422	41.1 (34.0)	39.5 (31.6)	0.4318	40.3 (32.9)
Physical activity level, n (%)			0.1376			0.4965			0.8412	
None	76 (13.6)	57 (9.9)		71 (12.7)	62 (10.8)		67 (11.6)	66 (11.9)		133 (11.7)
1-2 times per week	176 (31.4)	178 (31.0)		168 (30.0)	186 (32.4)		177 (30.5)	177 (31.9)		354 (31.2)
≥3 times per week	309 (55.1)	339 (59.1)		322 (57.4)	326 (56.8)		336 (57.9)	312 (56.2)		648 (57.1)

Legend: SD (standard deviation), BMI (Body Mass Index), *Comorbidity score by self-administered questionnaire51, #25-hydroxyvitamin D concentrations; SPPB (Short physical performance battery), IQR (interquartile range), DHA (Docosahexaenoic acid), EPA (Eicosapentaenoic acid); MET (metabolic equivalent of task)

zero positive items were classified as robust, and participants with one or two positive items were classified as being pre-frail, while participants with three or more positive items were classified as frail according to the definition by Fried et al (20). For the present study, robust participants at baseline who became pre-frail at any time point over the follow-up but who did not become frail were considered as pre-frail. Participants who were robust at baseline and became frail at any time point over the follow-up were considered as frail.

Assessment of biomarkers

As described previously, DSM Nutritional Products R&D Analytics performed 25-hydroxyvitamin D measurements and the Research Toxicology Center performed polyunsaturated fatty acid measurements (EPA and DHA) by sensitive and selective assays based on liquid chromatography coupled to a mass spectrometry detection system at baseline and at 12, 24, and 36 months (29). Mass spectrometry detection systems were monitored with standard, quality control, and human National Institute of Standards and Technology plasma reference samples. In accordance with the DO-HEALTH main paper, vitamin D repleteness was defined as serum 25-hydroxyvitamin D levels ≥20 ng/mL, vitamin D deficiency as serum 25-hydroxyvitamin D levels <20 ng/mL and severe deficiency as serum 25-hydroxyvitamin D levels <12 ng/mL (29).

Statistical analysis

The analytic dataset was a subset of DO-HEALTH participants who were identified as robust at baseline (n=1137). Baseline characteristics of the study population are described overall and by treatment group. Normally distributed continuous variables are presented as means and standard deviations (SD) and non-normal variables as median and inter quartile range (IQR). Categorical variables are presented in frequencies and percentages. Differences between treated and non-treated participants at baseline were tested using the Wilcoxon rank sum test, t-test or chi-square test, for non-normal, normal and categorical variables, respectively.

With frailty status being coded as robust/pre-frail/frail, and in violation of the proportional odds assumption (p=0.004), we fit a multinomial logistic regression model comparing 1) participants who became pre-frail at any time point over the three-year follow-up vs. participants who remained robust over three years; and 2) participants who became frail at any time point over the three-year follow-up vs. participants who

remained robust over three years.

We first tested the interaction effect of the treatment groups. Neither the three-way treatment interaction of vitamin D3*omega-3s*SHEP (p=0.998) nor the three two-way interaction effects of vitamin D3*omega-3s (p=0.27), vitamin D*SHEP (p=0.34), and omega-3s*SHEP (p=0.38) were significant in the multinominal logistic regression model. Therefore, the main effects of treatments vitamin D3, omega-3s, and SHEP were included in the models.

To test the individual and combined effects of study treatments, we adjusted for the following randomization stratification variables: age, sex, low-trauma falls (yes/no) during the 12 months preceding the randomization day and study site.

Results are presented as odds ratios and 95% confidence intervals. The type I error rate was set at 5%. All statistical analyses were performed using SAS v9.4 (SAS Institute, Inc., Cary, NC, United States).

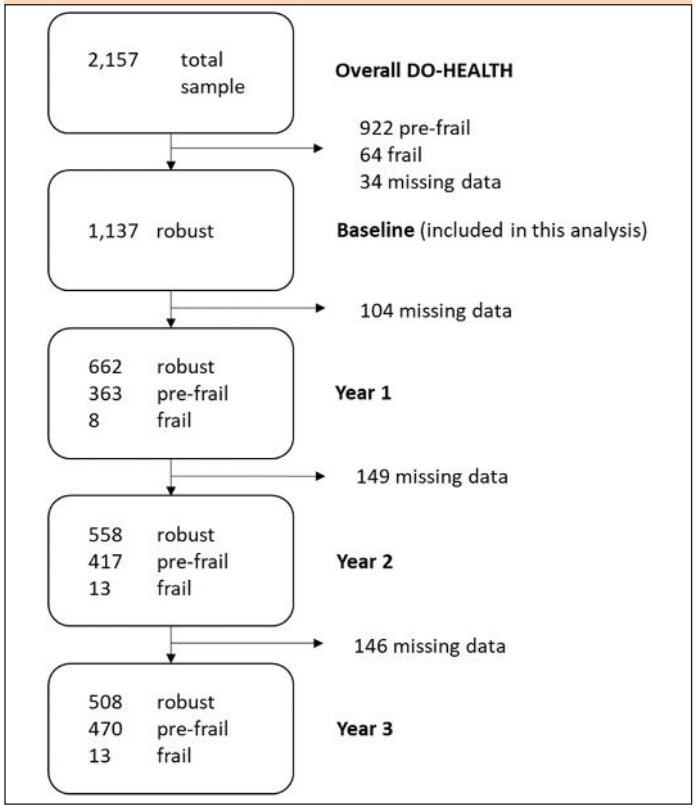
Results

Of the 2,157 DO-HEALTH participants, 1,137 were robust at baseline and were included in this analysis. Of those, 56.5% were women, mean age was 74.3 (±4.0) years, mean BMI was 26.1 kg/m^2 (±4.0) and 432 (38%) were living alone. Participants reported a mean physical activity volume of 40.3 (±32.9) metabolic equivalent of task-hours (MET-hrs) per week and a mean physical performance score of 11.3 points (±1.1) on the SPPB (score range: min. 0; max. 12 points). Upon enrolment, mean serum 25-hydroxyvitamin D concentration was 23.0 ng/mL while 38.0% of participants were vitamin D deficient with 25-hydroxyvitamin D levels below 20 ng/mL. In all, baseline characteristics of the treatment and non-treatment groups were balanced. Table 1 summarizes the baseline characteristics of the included participants, overall and by treatment group. With regard to adherence, 75.2% of participants took at least 80% of their total study pills and 54.5% of participants performed their exercise program at least two times per week.

Odds of becoming pre-frail by treatment group

Of the 1,137 robust participants at baseline, 691 (61.2%) became pre-frail over the three-year follow-up (Figure 1). Adjusting for age, sex, prior falls, and study site, the odds ratios (OR, 95% CI) of becoming pre-frail over the 3 year follow-up comparing the individual treatment effects were 0.81 (0.62-1.07; p=0.13) for receiving vitamin D3 vs. no vitamin D3, 0.84 (0.64-1.11; p=0.22) for receiving omega-3s vs. no omega-3s, and 0.89 (0.67-1.16; p=0.38) for receiving SHEP vs. no SHEP. In regard to the combined treatment effects, the odds ratios (OR, 95% CI) of becoming pre-frail over the 3 year follow-up were 0.69 (0.46-1.01; p=0.06) for receiving vitamin D3 plus omega-3s vs. no vitamin D3 and omega-3s, 0.75 (0.51-1.10; p=0.14) for receiving omega-3s plus SHEP vs. no omega-3s and SHEP, 0.72 (0.49-1.06; p=0.10) for receiving vitamin D3 plus SHEP vs. no vitamin D3 and SHEP, and 0.61 (0.38-0.98; p=0.04) for receiving all 3 treatments combined vs. placebo (Figure 2).

Figure 1. Flow chart of included participants



For this analysis we included only participants considered robust at baseline from the total DO-HEALTH study population according to the applied frailty phenotype

Odds of becoming frail by treatment group

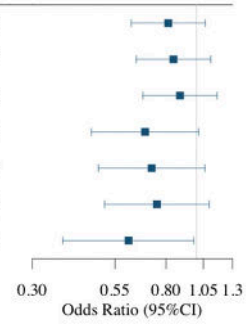
Over the three-year follow-up, 29 (2.6%) participants who were robust at baseline became frail. Given the small incidence of frailty in this generally healthy study population, there was limited power to test treatment effects for this outcome. Adjusting for age, sex, prior falls, and study site, the odds ratios (OR, 95% CI) of becoming frail over the three-year follow-up comparing the individual treatment effects were 1.19 (0.52-2.75; p=0.68) for receiving vitamin D3 vs. no vitamin D3, 0.71 (0.31-1.63; p=0.41), for receiving omega-3s vs. no omega-3s, and 2.19 (0.92-5.24; p=0.08) for receiving SHEP vs. no SHEP. In regard to the combined treatment effects, the odds ratios (OR, 95% CI) of becoming frail over the 3 year follow-up were 0.84 (0.26-2.72; p=0.77) for receiving vitamin D3 plus omega-3s vs. no vitamin D3 and omega-3s, 1.54 (0.48-4.95; p=0.46) for receiving omega-3s plus SHEP vs. no omega-3s and SHEP, 2.61 (0.78-8.74; p=0.12) for receiving vitamin D3 plus SHEP vs. no vitamin D3 and SHEP, and 1.85 (0.44-7.69; p=0.40) for receiving the 3 treatments compared to control (placebo for the supplements and control exercise) (Figure 3).

Discussion

In this three-year, double-blind, randomized controlled trial among 1,137 older adults who were robust at baseline, the combined interventions of daily supplemental 2000 IU vitamin

Figure 2. Treatment effects on the odds of becoming pre-frail during the follow-up

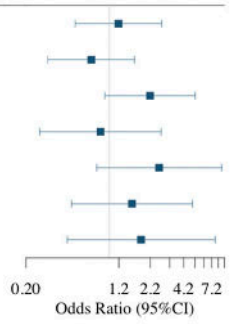
Treatments	Incidence in Treatment Group	Incidence in Control Group	Odds Ratio (95%CI)	P-values
Vit D vs No Vit D	333/528 (63.1%)	363/535 (67.9%)	0.81 (0.62-1.07)	p=0.134
Omega-3 vs No Omega-3	334/520 (64.2%)	362/543 (66.7%)	0.84 (0.64-1.11)	p=0.221
SHEP vs No SHEP	343/540 (63.5%)	353/523 (67.5%)	0.89 (0.67-1.16)	p=0.379
Vit D+ Omega-3 vs No VitD + Omega-3	160/251 (63.8%)	189/266 (71.1)	0.69 (0.46-1.01)	p=0.059
Vit D + SHEP vs No VitD + SHEP	166/264 (62.9%)	186/259 (71.8%)	0.72 (0.49-1.06)	p=0.097
Omega-3 + SHEP vs No Omega-3 + SHEP	164/266 (61.7%)	183/269 (68.0%)	0.75 (0.51-1.10)	p=0.135
Vit D + Omega-3 + SHEP vs Placebo	80/126 (63.5%)	96/130 (73.9%)	0.61 (0.38-0.98)	p=0.041



Vit D = Vitamin D supplementation, Omega-3 = Omega-3 fatty acids supplementation, SHEP = simple home exercise program

Figure 3. Treatment effects on the odds of becoming frail during the follow-up

Treatments	Incidence in Treatment Group	Incidence in Control Group	Odds Ratio (95%CI)	P-values
Vit D vs No Vit D	18/528 (3.4%)	11/535 (2.1%)	1.19 (0.52-2.75)	p=0.679
Omega-3 vs No Omega-3	13/520 (2.5%)	16/543 (3.0%)	0.71 (0.31-1.63)	p=0.413
SHEP vs No SHEP	20/540 (3.7%)	9/523 (1.7%)	2.19 (0.92-5.24)	p=0.078
Vit D+ Omega-3 vs No VitD + Omega-3	9/251 (3.6%)	7/266 (2.6%)	0.84 (0.26-2.72)	p=0.773
Vit D + SHEP vs No VitD + SHEP	12/264 (4.6%)	3/259 (1.2%)	2.61 (0.78-8.74)	p=0.119
Omega-3 + SHEP vs No Omega-3 + SHEP	11/266 (4.1%)	7/269 (2.6%)	1.55 (0.48-4.97)	p=0.464
Vit D + Omega-3 + SHEP vs Placebo	7/126 (5.6%)	3/130 (2.3%)	1.85 (0.44-7.69)	p=0.401



Vit D = Vitamin D supplementation, Omega-3 = Omega-3 fatty acids supplementation, SHEP = simple home exercise program

D3 plus daily 1g marine omega-3 plus SHEP had significant benefits with regard to the prevention of pre-frailty. However, this benefit was not significant for the 3 treatments individually, highlighting the importance of the additive effect of all three preventive strategies.

Given that the DO-HEALTH participants were generally healthy, physically active and robust, only few (2.6%) developed frailty over the 3-year follow-up. Thus, we were underpowered to address the odds of frailty by treatment group, and were not able to detect beneficial treatment effects extending from pre-frailty to frailty.

The association of vitamin D and frailty status has been investigated in multiple observational studies, demonstrating that low vitamin D levels are associated with increased risk of becoming frail (34). However, to the best of our knowledge, no prior randomized clinical trial in generally healthy and largely vitamin D replete community dwelling older adults has investigated the effect of vitamin D3 supplementation for the prevention of pre-frailty as a predefined primary outcome. Therefore, DO-HEALTH contributes important data to the existing literature, exhibiting a beneficial effect of 2,000 IU vitamin D3 supplementation on the odds of becoming pre-frail when combined with omega-3 and SHEP.

For omega-3s, to our knowledge, no randomized controlled trials have investigated the effect of omega-3 supplementation on the development of pre-frailty as a primary outcome. In an analysis of participants in the InCHIANTI study, higher physical function was associated with higher levels of n-3 PUFAs (35). The Multidomain Alzheimer Preventive Trial (MAPT), including over 1,500 community-dwelling older adults investigating omega-3s supplementation and a multi-domain intervention (physical activity, cognitive training, and nutritional advice), alone or in combination, compared with placebo, did not find a positive signal in regard to the development of frailty by supplemental omega-3s over a 3 year period (36). However, this trial did not report on the prevention of the at risk-state of pre-frailty and included participants with memory complaints and functional impairments. Therefore, we are limited in our ability to make direct comparisons to other study populations of the potentially beneficial effect of omega-3s combined with the other treatments to the generally healthy DO-HEALTH population.

In regard to exercise interventions, systematic reviews investigating the impact of exercise in non-frail, pre-frail and frail community dwelling older adults already suggested its beneficial effect on frailty progression. However, there still remains some uncertainty regarding the optimal duration, frequency, and type of exercise (28, 37, 38). In a recent randomized controlled trial among 100 frail participants, a multicomponent exercise program (including proprioception, aerobic, strength, and stretching exercises for 65 minutes, 5 days per week, for 24 weeks) led to a reduction in frailty level of 30% in the intervention group (39). In our study, the unsupervised SHEP program of DO-HEALTH for 30 minutes 3 times per week for 3 years had no significant effect on the incidence of pre-frailty and frailty individually, though

it significantly reduced the odds of becoming pre-frail when combined with the other treatments.

The combined effect of the three DO-HEALTH interventions on the prevention of pre-frailty may be explained by their complementary influences on the complex pathophysiological pathways that are linked to the development of frailty, including muscle function, chronic inflammation, cardiovascular health and immune system regulations even among robust, physically active and generally healthy older adults (6, 40, 41). Therefore, our findings of a combined effect might be explained by additive ergogenic properties of the three interventions (42) and their potential effects on the reduction of chronic low-grade inflammation (43-45). In particular, vitamin D has been described as independently and inversely associated with interleukin (6), suggesting a potential anti-inflammatory role in older individuals (46). Further, anti-inflammatory properties have also been described for omega-3s (6). Besides their role in the prevention of cardiovascular diseases, it is suggested they alter the lipid profile, producing an anti-inflammatory effect by improving vascular endothelial function and insulin sensitivity (47). In addition to the well-established influence of vitamin D on musculoskeletal health (48), mechanistic studies also support the involvement of vitamin D in the development of cardiovascular diseases (49). Lastly, besides the known effects of exercise on cardiovascular health, its anti-inflammatory effects might be mediated by mechanisms on cytokine regulation including interleukin 6, especially in older adults (44, 50).

Our study has several strengths. Primarily, this is the first multicenter randomized clinical trial investigating the individual and combined effects of supplemental vitamin D3, omega-3s and a SHEP on the primary prevention of pre-frailty in a large sample of generally healthy and community dwelling European older adults over a follow-up of 3 years. In addition, frailty status was a predefined secondary outcome in the study protocol, captured by a standardized procedure, and assessed by trained study personal according to a strict protocol at all sites.

However, a few limitations can be acknowledged. First, this is a secondary analysis of the DO-HEALTH trial, designed to investigate the effect of the three interventions in regard to 6 primary outcomes (change in systolic and diastolic blood pressure, physical performance, cognitive function, and incidence rates of non-vertebral fractures and infections over 3 years). In addition, the relatively good health and low incidence of frailty, despite the DO-HEALTH inclusion criteria containing prior falls, resulted in the analysis of overt frailty as a primary outcome to be underpowered. Furthermore, supplemental vitamin D3 800 IU/d was allowed in all participants, and SHEP was randomized against control exercise (joint flexibility 3x 30 min/wk.).

Overall, our results suggest a beneficial effect of supplemental vitamin D3, omega-3s and an unsupervised SHEP in combination for the prevention of pre-frailty in generally healthy older adults. Further investigation is warranted to determine whether the combined benefits of the DO-HEALTH interventions are superior compared to a healthy and active lifestyle including a comparative amount of dietary omega-3s and regularly physical exercise in this target group. At the same time, the potential benefits of the combined daily supplementation of 2,000 IU of vitamin D3, 1g of marine omega-3s and a SHEP in regard to the prevention of pre-frailty and frailty should be further investigated in additional clinical trials including the in-depth study of the complex underlying mechanisms on the molecular level.

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Ethical standards: The DO-HEALTH study was approved by ethics and regulatory agencies of all 5 countries and the study protocol has been previously published (30). A data and safety monitoring board oversaw the study.

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