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DO-HEALTH: Vitamin D3 - Omega-3 - Home exercise - Healthy aging and longevity trial - Design of a multinational clinical trial on healthy aging among European seniors

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ABSTRACT

DO-HEALTH is a multi-center clinical trial among 2157 community-dwelling European men and women age 70 and older. The 2x2x2 randomized-control factorial design trial tested the individual and additive benefit, as well as the cost-effectiveness, of 3 interventions: vitamin D 2000 IU/day, omega-3 fatty acids 1000 mg/day (EPA + DHA, ratio 1:2), and a 30-minute 3 times/week home exercise (strength versus flexibility). Each treatment tested has shown considerable prior promise from mechanistic studies, small clinical trials, or large cohort studies, in the prevention of common age-related chronic diseases, but definitive data are missing. DO-HEALTH will test these interventions in relation to 6 primary endpoints (systolic and diastolic blood pressure, non-vertebral fractures, Short Physical Performance Battery score, the Montreal Cognitive Assessment, and risk of infections), plus several secondary endpoints explored in ancillary studies (i.e. rate of any falls and injurious falls, joint pain, oral health, quality of life, and incident frailty). As the 3 interventions have distinct mechanisms of action for each of the 6 primary endpoints, a maximum benefit is expected for their additive benefit as a "multi-modal" intervention. The trial duration is 3 years with in-person contacts with all participants at 4 clinical visits and by quarterly phone calls. Baseline and follow-up blood samples were collected in all participants to measure changes in 25-hydroxyvitamin D and poly-unsaturated fatty acid concentrations. Our objective was to test interventions that are expected to promote healthy aging and longer life expectancy and that can be easily and safely implemented by older community-dwelling adults.

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[1] In Memory of Dieter Felsenberg, a passionate scientist in clinical muscle and bone research.

[2] DO-HEALTH Research Group is listed in the Appendix 1.

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1. Introduction

In Europe, the number of adults age 65 years and older is expected to be more than double in the coming 40 years. Specifically, for the five DO-HEALTH countries, the UN Population Division estimated in 2019 that the population age 65 and older will grow by 78% in Switzerland, 61% in Austria, 32% in Germany, and 41% in France between 2020 and 2060 [1]. Concomitantly the number of older adults with age-related chronic diseases is increasing [2,3]. Thus, therapeutic interventions that are effective, affordable, and well-tolerated in the prevention of chronic conditions at older ages are urgently needed and have the potential for major impact on public health. Among the most promising interventions that meet these requirements are vitamin D, marine omega-3 fatty acids (n-3 s), and physical exercise. However, their individual and combined effects in older adults have yet to be confirmed in a large clinical trial [4–6].

About half of older adults worldwide have low vitamin D levels (<50 nnmol/mL or < 20 ng/mL) [7]. This is in part explained by their skin being less able to synthesize vitamin D in response to solar radiation, less sun exposure due to sedentary life style, and to sun avoidance in older adults [8–12]. Also, dietary sources of vitamin D are rare and largely limited to fatty fish, fish liver oil, egg yolk, and mushrooms [119]. Similarly, low n-3 s intake and lack of exercise are acknowledged public health concerns, especially in the older adult population [2,3,13]. However, despite the recognition of frequent inadequacy of vitamin D, n-3 s, and exercise, broad public recommendations cannot currently be justified because definitive data on health benefits and risks of the 3 interventions individually and in combination are lacking.

Meta-analyses of randomized clinical trials (RCTs) have reported protective effects of n-3 s against cardiovascular disease [14], cognitive decline [15,16], and bone turnover markers [17]. However, several meta-analyses found no significant associations with coronary heart disease [18,19] and cognitive function [20,21]. Vitamin D has also been associated with inconsistent findings for all-cause all causes of mortality [22–24], fractures (particularly when combined with calcium) [25–27], and respiratory infections [28,29] in meta-analysis of RCTs. Physical exercise has been associated with reduced risk of community-acquired pneumonia [30] and cognitive decline [31], improvement in blood pressure [32] and functional performance [33]. However, evidence of the benefits of physical exercise on risk of fractures is lacking from randomized controlled trials.

DO-HEALTH was designed to establish evidence for the role of vitamin D, n-3 s, and a simple home based strength exercise program (SHEP), both individually and as a combined intervention, in chronic conditions prevention at older ages, for 6 primary endpoints (systolic and diastolic blood pressure change, non-vertebral fractures, the Short Physical Performance Battery (SPPB) [34], the Montreal Cognitive Assessment (MoCA) [35], and infections), addressing 5 health domains (cardiovascular health, bone health, muscle health, brain-health, and immunity), supported and extended by a series of secondary and exploratory endpoints.

2. Methods

2.1. Study design and hypothesis

DO-HEALTH is a 3-year randomized, double-blind, placebo-controlled, 2x2x2 factorial design trial that tested 3 primary treatments:

(1) Dietary supplement of 2000 IU vitamin D per day compared to placebo
(2) Dietary supplement of 1 g of n-3 s (eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA), ratio 1:2, from marine algae) compared to placebo.
(3) SHEP (Strength) of 30 min 3 times a week compared to a control exercise program (Flexibility) 30 min 3 times a week.

Our hypotheses are that vitamin D, n-3 s, and SHEP, alone or in combination, can improve six primary endpoints: reduction in systolic and diastolic blood pressure (cardiovascular health), reduction in non-vertebral fracture rates (bone health), increase in SPPB scores (muscle health), increase in MoCA scores (brain-health), and reduction in infection rates (immunity). Therefore, all analyses will test the effect of each strategy individually and their combinations (Fig. 1). The factorial design was chosen to increase power by taking advantage of our assumption that vitamin D, n-3 s, and the SHEP have distinct mechanisms of action and therefore may have an additive effect on the study endpoints. If the analyses show that the effects are not additive, then all effects can still be estimated without bias, but with reduced power.

The trial was performed at 7 recruitment centers located in 5 European countries: Switzerland (University of Zurich, Basel University Hospital, Geneva University Hospital), France (University of Toulouse Hospital Center), Germany (Charité Berlin), Portugal (University of Coimbra), and Austria (Innsbruck Medical University).

2.2. Endpoints

Fig. 2 lists the schedule of assessment of the 6 DO-HEALTH primary endpoints (Fig. 2A), and all secondary endpoints (Fig. 2B), as well as health-care utilization data collected at each time point. Supplementary Table 1 describes all endpoints assessed in DO-HEALTH (primary, secondary, exploratory, and biomarkers). Supplementary Table 2 describes all questionnaires used in DO-HEALTH at baseline, 12, 24 and 36 months of follow-up.

2.3. Primary endpoints assessments

The DO-HEALTH trial addressed 6 primary endpoints in 5 domains (Fig. 2):

2.3.1. Cardiovascular

Systolic and diastolic blood pressure changes were assessed by blood pressure measurements after 5-min rest in a seated position following a standardized protocol validated in a DO-HEALTH pilot trial [37].

2.3.2. Bone

Incidence of non-vertebral fractures. All fracture events were confirmed by X-ray reports or medical records that describe an X-ray report of the fracture or mention the repair of the fracture.

2.3.3. Muscle

Functional decline was assessed with the Short Physical Performance Battery (SPPB) [34], which is an objective assessment tool for evaluating lower extremity function in older persons. It was developed by the National Institute on Aging and has been validated extensively in epidemiologic studies and in intervention trials [34]. The SPPB is a brief performance-based test that includes walking speed, repeated chair stands, and a balance test. Its three components are scored between 0 and 4, with 4 indicating the highest level of performance, and are summed up to yield an overall score.

2.3.4. Brain

Cognitive decline was assessed using the Montreal Cognitive Assessment (MoCA) [35]. The MoCA is more structured and covers more cognitive domains than the Mini Mental State Examination (MMSE) and has been found to be more sensitive than MMSE with respect to mild cognitive impairment [35,38–41].

2.3.5. Immunity

Rate of any infection. Upon each contact, the participant was asked whether any infection with or without fever had occurred and whether and when a vaccination was performed (i.e. flu vaccination). When a participant experienced an infection, a detailed infection questionnaire

developed in two pilot trials to DO-HEALTH [42,43] was administered. The questionnaire elicits information about symptoms, treatment, primary doctor contact and medical assessments. Every case of infection was verified by an independent physician using all available information (symptoms, treatment, and medical records).

2.4. Secondary endpoints assessments

DO-HEALTH also assessed additional secondary endpoints that supported the primary endpoints and extended to other organ systems (Fig. 2).

2.4.1. Cardiovascular

- Risk of incident hypertension. Among participants without a diagnosis of hypertension, the first occurrence of SBP ≥140 mmHg or DBP ≥90 mmHg on a study visit qualified as incident hypertension. In addition, a chart-based diagnosis of incident hypertension and/or a report of new use of an antihypertensive drug qualified as incident hypertension.

2.4.2. Bone

- Incidence of hip fractures. Diagnosis of a hip fracture was confirmed by X-ray report or medical record mentioning an X-ray report or fracture repair. Hip fractures included femoral neck and trochanteric fractures.
- Incidence of vertebral fractures (vertebral morphometry). Diagnosis of a vertebral fracture was based on iDXA vertebral morphometry assessment among 1492 participants at the four recruiting centers equipped with Lunar iDXA, GE Healthcare machines (Zurich, Berlin, Toulouse, Coimbra). All vertebral bodies of the thoracic and the lumbar spine were categorized as normal or deformed. Deformities were classified as: *mild* (grade 1, 20–25% reduction in either anterior or middle height relative to posterior height of the same vertebral body, or reduction of posterior height relative to posterior height of adjacent vertebral bodies); *moderate* (grade 2, 26–40% reduction in any height); and *severe* (grade 3, >40% reduction in any height) deformities. All vertebral deformities were classified according to etiology (for example osteoporotic, traumatic, degenerative, etc.). The assessment of prevalent and incident vertebral deformities based on iDXA morphometry was blinded to treatment and performed at one specialized center (Berlin). Additionally, we assessed the incidence of mild to severe vertebral deformities (e.g. biconcave, compression, and wedge), and the incidence of moderate to severe vertebral deformities due to osteoporosis.
- Incidence of total fractures. Sum of any new non-vertebral and any new vertebral fracture (mild to severe deformity) based on vertebral morphometry assessment in the subset of 1492 participants at the four recruiting centers equipped with Lunar iDXA, GE Healthcare machines (Zurich, Berlin, Toulouse, Coimbra). Bone mineral density at the spine and hip based on iDXA measurements was assessed in 1492 participants at four recruiting centers equipped with Lunar iDXA, GE Healthcare machines. The measurements were performed during each clinical visit. Both (right and left) proximal femurs were scanned at baseline. At follow-up, only the hip on the side that had the lower bone mineral density value of the total femur (hip) at baseline was measured. If the hip side with the lower baseline bone mineral density became unsuitable for DXA measurement (e.g. due to fracture or endoprosthesis during the course of the study), the other side was analyzed.

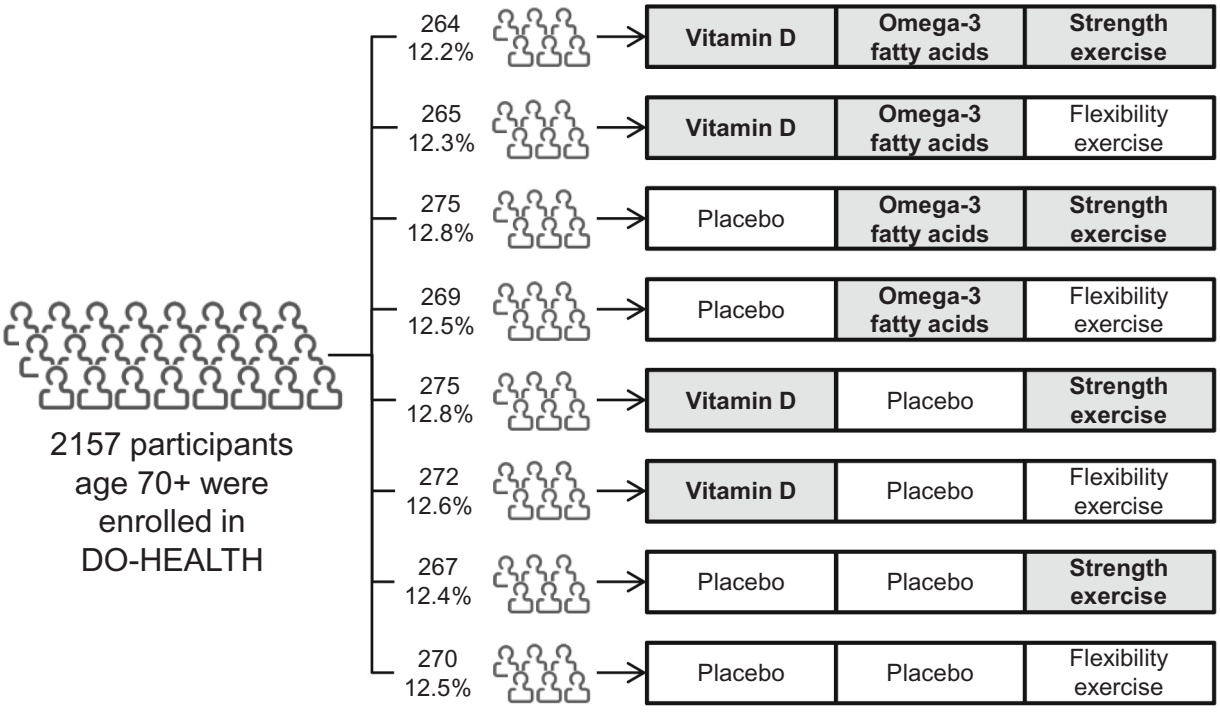

Fig. 1. DO-HEALTH study schema.
Legend: Participants were randomized in 8 arms and followed over 3 years (36 months) in 4 clinical visits (Baseline, 12-month, 24-month, 36-month visits) and in 9 three-monthly phone calls (3-, 6-, 9-, 15-, 18-, 21-, 27-, 30-, 33-month phone calls).

2.4.3. Muscle

- Rate of falling (rate of any low trauma-, injurious falls, number of participants who fell with and without injury). Falling was defined as unintentionally coming to rest on the ground, floor, or other lower level (coming to rest against furniture or a wall is not considered a fall). Incident falls were ascertained with a questionnaire to assess the circumstance of each fall and associated injuries.
- Reaction time and grip strength. Reaction time was assessed with repeated chair stands (5 repeats as part of the SPPB [34]), and muscle strength was assessed with a validated grip strength protocol using Martin vigorimeter [44].
- Muscle mass in the upper and lower extremities. Muscle mass (appendicular lean mass/height^2) was measured by iDXA in 1492 participants at the four recruiting centers equipped with Lunar iDXA, GE Healthcare machines (Berlin, Coimbra, Toulouse, and Zurich).
- Musculoskeletal pain was assessed using McGill pain map [45].
- Dual tasking 10 m gait speed was assessed by comparing normal gait speed against gait speed if combined with a simple cognitive task over a distance of 10 m. Gait speed was measured with a stop watch under two conditions: "single task," usual walking at preferred speed and using usual walking aid, and "dual task", walking while subtracting serial twos from a predefined number (baseline: from 50; 12 months: from 100; 24 months: from 70; 36 months: from 80) under the same conditions– without explicit instructions regarding prioritization (walking or counting) [46]. The difference between the two times was evaluated for this endpoint. At the study center in Basel, dual tasking gait speed was measured using the GAITRite electronic walkway system in addition.

2.4.4. Brain

- Mental health decline and incidence of depression were assessed using an *excerpt* from the Geriatric Depression Scale (GDS) [47,48].
- Dual tasking gait variability was assessed in 250 participants by gait analyses and dual task assessments (GAITRite® Platinum, CIR Systems, PA, USA) in one recruitment center (Basel University Hospital). Participants performed 4 walking tasks with and without cognitive challenge [36].

2.4.5. Immunity

- Rates of any upper respiratory infection, incident flu-like illness, incident severe infections that lead to hospital admission. This was based on an infection protocol (see primary endpoint). Infections and type of infection that lead to hospital admission were confirmed by medical records.

2.4.6. Cartilage

All participants were evaluated for prevalent knee osteoarthritis (OA) at baseline according to the modified American College of Rheumatology (ACR) criteria [49]. According to the modified ACR clinical criteria, prevalent and incident symptomatic knee OA were diagnosed in participants who reported knee pain on most days of the preceding week plus at least 3 of 6 criteria:

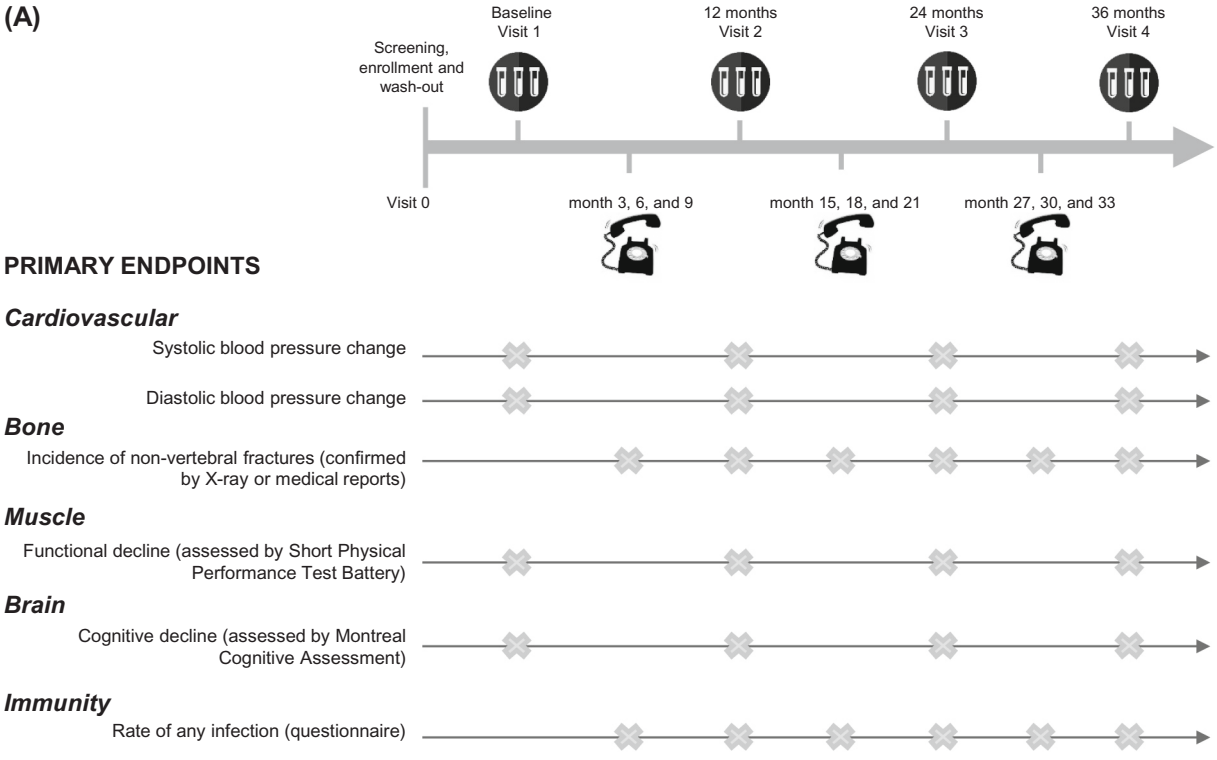

Fig. 2. Schedule of assessment of the primary (A) and secondary (B) endpoints.
Legend: In DO-HEALTH, participants were followed over 3 years (36 months). After the prescreening phone call, participants had 4 clinical visits, including baseline, and 9 phone calls.
*assessments performed with 1502 participants, 4 recruiting centers equipped with Lunar iDXA, GE Healthcare machines (Berlin, Coimbra, Toulouse, and Zurich).
**Dual tasking gait variability was assessed in 250 participants at baseline, 12, 24, and 36 months by gait analyses and dual task assessments (GAITRite® Platinum, CIR Systems, PA, USA) in one recruitment center (Basel University Hospital). Participants performed 4 walking tasks with and without cognitive challenge [36].
¶Quality of life was evaluated at baseline, and every 6 months afterwards (during 6-, 18-, 30-month phone calls, and at 12-, 24-, and 36-month clinical visits).

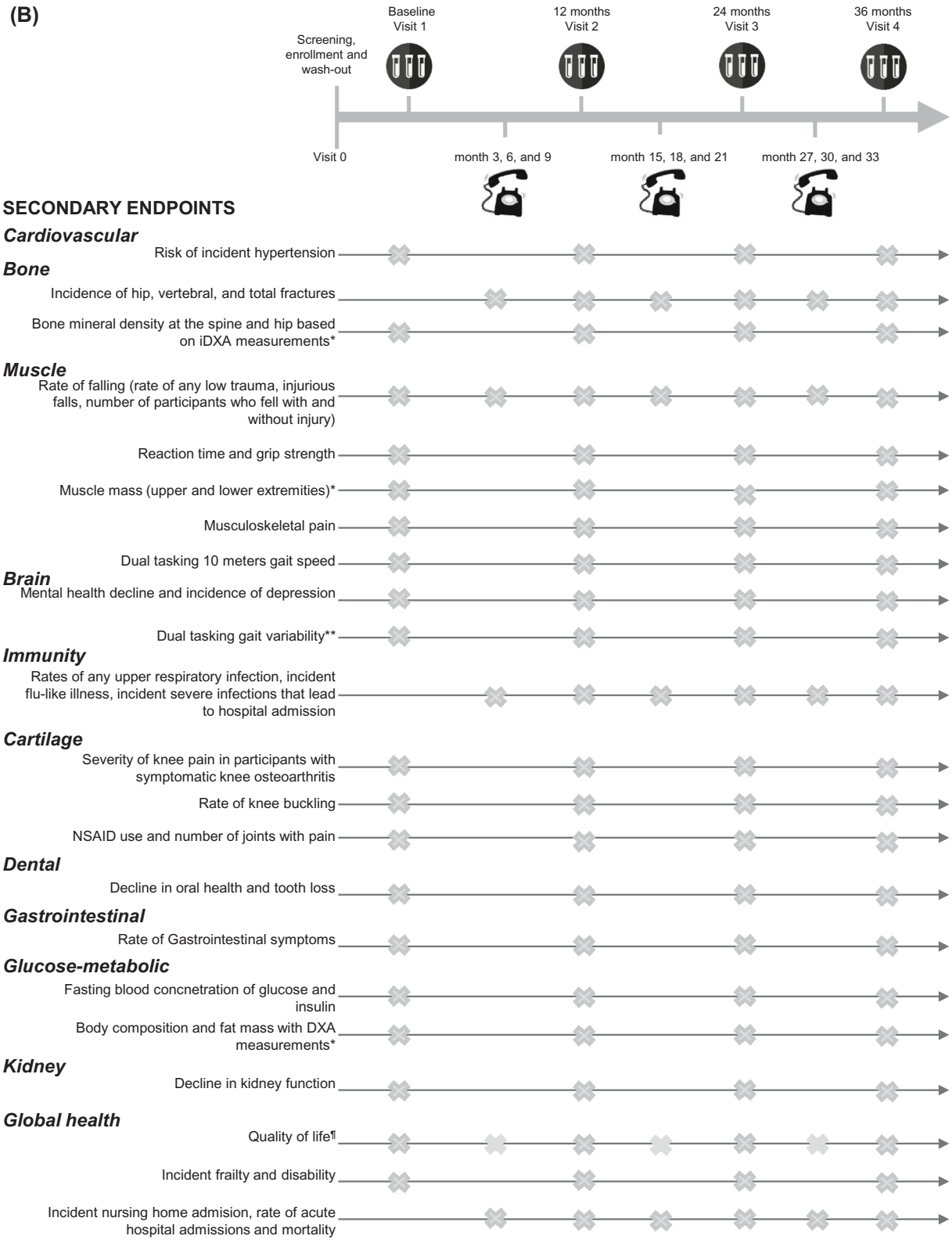

Fig. 2. (*continued*).

1) Crepitus based on physical exam performed by the study medical doctor
2) Age 50+
3) Morning stiffness <30 min
4) Bony tenderness based on physical exam performed by the study medical doctor
5) Bony enlargement based on physical exam performed by the study medical doctor
6) Palpable warmth based on physical exam performed by the study medical doctor

The ACR criteria for knee OA were modified for DO-HEALTH. The

bony enlargement criterion was excluded due to its poor reproducibility.

- Severity of knee pain was assessed with the Knee injury and Osteoarthritis Outcome Score (KOOS [50]).
- Rate of knee buckling was assessed with a comprehensive and validated questionnaire [51].
- Nonsteroidal anti-inflammatory drugs (NSAID) use to control knee pain was assessed in all participants by standardized questionnaire. Additionally, participants were asked to report the average number of days per month they took NSAIDs.
- Number of joints with pain was assessed in all participants with a joint map.

2.4.7. Dental

- Decline in oral health was assessed with the Geriatric Oral Health Assessment Index (GOHAI [52]) questionnaire.
- Tooth loss was assessed by tooth count by the study medical doctor including all teeth (own and all) and was validated with tooth prints at one recruitment center (University of Zurich).

2.4.8. Gastro-intestinal

- Gastro-intestinal symptoms were assessed using Rome III diagnostic questionnaire for the adult functional gastro-intestinal disorders [53].

2.4.9. Glucose-metabolic

- Fasting blood concentration of glucose and insulin. Insulin sensitivity and beta cell function derived from indices of fasting glucose and insulin concentrations were calculated using quantitative insulin-sensitivity check index (QUICKI), and the HOMA index [54].
- Body composition and fat mass were measured by iDXA in 1502 participants recruited by four centers equipped with Lunar iDXA, GE Healthcare machines. We assessed trunk and upper and lower extremity fat mass.

2.4.10. Kidney

- Decline in kidney function was measured by blood creatinine levels and estimated glomerular filtration rate with the Cockcroft-Gault formula [55].

2.4.11. Global health

- Quality of life was evaluated using the EQ5D-3 L questionnaire.
- Incident frailty was assessed with the Survey of Health, Aging and Retirement in Europe Frailty Instrument (SHARE-FI) [56]. SHARE-FI closely resembles the traditional Fried's variables [57,58], and defines frailty condition by evaluating exhaustion, weight loss, slowness, low activity and weakness (the latter assessed by measuring handgrip strength).
- Incident disability regarding activities of daily living was measured by the PROMIS-HAQ [59]. We assessed the risk of at least one new limitation in 20 activities of daily living (we assessed having at least some difficulty to do the activity / unable to do the activity).
- Incident nursing home admissions, rate of acute hospital admissions and mortality were assessed every 3 months. Mortality was confirmed by medical record or death certificate; cause of death was established from medical records or death certificate and classified as follows: cardiovascular, cancer, severe infections, or other.

2.5. Diet assessment for subgroup analyses (calcium/protein intake)

We developed the electronic DO-HEALTH Food Frequency Questionnaire (FFQ) targeted at older adults. The instrument was created to be consistent across the DO-HEALTH countries and the DO-HEALTH study languages (German, French, and Portuguese). The FFQ structure is based on food groups (the so-called European Food Groups, EFGs). This feature makes the tool easily used in culturally different populations, as compared to FFQs organized e.g. by meal patterns. Each food group includes several distinct food items. For each item the frequency of consumption, in terms of a standard portion, is assessed: rarely/never; 1–3 per month; 1 per week; 2–4 per week; 5–6 per week; 1 per day; 2–3 per day; 4–5 per day; 6+ per day. Additionally, for each fruit or vegetable item, we allow subjects to indicate if the consumption is only in season. Diet assessment by FFQ was performed at baseline and year 3.

2.6. Economic model

DO-HEALTH will assess the cost-benefit of the 3 interventions in a health economic model based on documented health care utilization. Health care utilization (direct medical and non-medical costs) was assessed in 3-month intervals (4 clinical visits and 9 follow-up phone calls) with a standardized questionnaire in all 2157 participants. The economic model will be a decision-analytic model along the trial based on original participant-level data from the DO-HEALTH. The overall goal is to evaluate (quality-adjusted) life years, costs and incremental cost effectiveness of vitamin D supplementation, n-3 s supplementation, and the SHEP in order to support healthy aging to increase healthy life expectancy. The six primary endpoints and number of falls (secondary endpoint) will be considered, and we will extract country-specific, officially reported aggregate cost data to impute overall healthcare costs by participant.

2.7. Direct data entry

DO-HEALTH developed a system that allowed participants themselves to directly enter much of the data. Running on standard computer tablets, questionnaires were presented using large, clear text and controls, with a simple and intuitive interface that received good reviews from the participants. The software gave immediate feedback on missing or inconsistent data, so that these issues could be corrected immediately, before the participants moved on. The same forms were available in all of the DO-HEALTH languages. Since WLAN access throughout the study centers was not guaranteed, data was stored locally on the tablets until the end of the day, at which point it was uploaded to the central server. The tablets were part of the overall software system which also allowed study staff to use PCs to enter data, check and resolve queries, randomize participants, schedule visits, allocate supplies, and check on the status of the study.

2.8. Study interventions

2.8.1. Vitamin D_3

Each active vitamin D capsule contained 1000 IU of Vitamin D_3, an oily liquid consisting of crystalline Vitamin D_3 (cholecalciferol) in medium chain triglycerides, stabilized with dl-α-tocopherol (vitamin E, 2.5 promille). As placebo, participants received capsules with (high oleic sunflower oil). High oleic sunflower oil was cold pressed from organic sunflower kernels, refined and deodorized. The appearance was clear, light yellow and liquid. Each participant took two capsules daily for a total dose of 2000 IU vitamin D/day or placebo.

2.8.2. Dose justification

The dose of 2000 IU vitamin D/day is in accordance with data from prior studies suggesting that 2000 IU of vitamin D is the required dose to

reach an average of 75 nmol/L of 25(OH)D previously suggested to be most desirable for fracture prevention [60–62]. In a prior study among hip fracture patients [63], 2000 IU vitamin D compared to 800 IU vitamin D/day reduced hospital readmission by 39%, fall-related injury by 60%, and severe infections by 90%. Further, 2000 IU vitamin D raised 25(OH)D levels to at least 75 nmol/L in over 90% of participants at 6 and 12 month follow-up, and was safe as demonstrated by repeated serum and urinary calcium excretion assessments [63]. Notably, we chose a daily administration of vitamin D in DO-HEALTH, as unphysiologically high intermittent bolus doses of vitamin D may be ineffective or have detrimental effects on falls [64,65] and fractures [66,67]. Similar to the U.S. Vital trial [68], we chose vitamin D_3 rather than D_2 in DO-HEALTH because vitamin D_3 is associated with higher increase in serum 25(OH)D concentrations and decreased mortality in older adults [22,69,70].

2.8.3. Safety

The safety of 2000 IU/day of vitamin D is further supported by our benefit-risk analysis wherein a safe upper intake level of 10,000 IU of vitamin D/day was estimated [62]. As all DO-HEALTH participants, according to current guidelines [71], were allowed to have an additional vitamin D intake of 800 IU/day, the maximum total intake of vitamin D was 2800 IU/day in the treatment group and 800 IU/day in the control group. These intakes are below the safe upper level of 4000 IU/day as defined by the Institute of Medicine [72].

2.8.4. We have not included calcium supplementation as a component of the intervention

At the higher dose of vitamin D intake, additional high dose calcium supplementation ($\geq$ 1000 mg), either alone or in combination with vitamin D, was not beneficial for fracture reduction [25,73]. However, since DO-HEALTH was designed, new evidence has emerged that the combination of calcium and vitamin D may be more effective at the skeleton than vitamin D alone [27]. In the participant instructions, we encouraged participants to meet calcium needs from dietary sources. A personal intake of calcium supplements up to 500 mg daily was allowed in DO-HEALTH.

2.9. Omega-3 fatty acids (n-3s)

Each active n-3 s capsule contained 500 mg of eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) in a ratio of 1:2. The formulation was an oily liquid containing at least 75% n-3 polyunsaturated fatty acids in the form of ethyl esters predominantly as EPA and DHA. It was stabilized with mixed tocopherols and ascorbyl palmitate. Rosemary extract was used as a processing aid. The placebo capsule contained a high oleic sunflower oil. Participants were instructed to take orally 2 study capsules daily for a total dose was 1000 mg of EPA and DHA or placebo. To reduce the risk of fishy after taste (and therefore risk of unblinding), we took several precautions: 1) we used n-3 s from algae, 2) all capsules had a gastric coating to be released only in the intestine, and 3) we instructed participants to take the capsules with dinner (or the preferred meal) and a cold drink (water or juice).

2.9.1. Dose justification

We chose 1 g of n-3 s daily as the treatment dose of n-3 s as this was beneficial in one secondary prevention trial for cardiovascular health [74]. Although the optimal ratio of EPA to DHA is unknown [75–77], we selected a 1:2 ratio of EPA to DHA. A total dose of 850 mg/day was used in the GISSI-Prevenzione Trial (EPA to DHA ratio, 1.2:1) [74] and AREDS (EPA to DHA ratio, 1.86:1), whilst a dose of 1.8 g/day of EPA was used in JELIS [78]. Ongoing trials are currently investigating higher doses n-3 s. VITAL tested a dose of 1 g/ day in a ratio of 1.3:1 of EPA to DHA [68], REDUCE-IT selected a dose of 4 g/day of icosapent ethyl, a highly purified ethyl ester of EPA [79], and STRENGTH, a dose of 1 g/ day of n-3 s without specifying the ratio [80]. Given that the average intake of EPA + DHA is 100 to 350 mg/d in many parts of Europe [81,82], the proposed intervention of 1 g/day is expected to increase the average participant's n-3 s intake by a factor of 3 to 10.

2.9.2. Safety

Health risks associated with fish oil are minimal and marine n-3 s doses of up to 3 g daily are considered safe. Although n-3 s have potential antithrombotic effects, systematic reviews of data from small, short-term trials suggest that n-3 s supplements do not increase the risk of clinically significant bleeding at doses less than 4 g daily, even in combination with anticoagulant interventions such as aspirin or warfarin [83,84].

2.10. Capsules stability

Quality control regarding stability testing was performed in detail by the manufacturer of the study capsules (DSM Nutritional Products) for each batch. Furthermore, the analytical services laboratory evaluated the stability of vitamin D3 and n-3 s over the course of 3 years at 25 °C in the soft gel capsules.

2.11. Simple home-based strength exercise program (SHEP)

A motivational and animated video was developed to instruct both the main exercise intervention (SHEP) as well as the control program (flexibility) in DO-HEALTH. Participants who wanted to step up the intensity of the program were invited to repeat the whole program, thus increasing the training time. The flexibility exercise program was designed to serve as a high-quality control expected to have no benefit on the endpoints tested in DO-HEALTH. In addition, paper versions of both programs were provided to participants. Both programs were demonstrated once during the baseline visit by an "instruction physiotherapist" not involved in the outcome assessment of DO-HEALTH, who also explained how to use the video, and the additional paper version. The physiotherapist also instructed the participant on the training strategy, e.g. to reduce repetitions or take a 1-week break if there was pain with exercise, and to call the recruitment center in case of any problems related to the program. Participants were asked to perform the home exercise program for 30 min 3 times a week.

2.11.1. Strength home-based exercise intervention program (SHEP)

SHEP consisted of the following exercises:

- Sit-to-stand (quadriceps strength training)
- One-leg stance (hip muscle strength training plus balance training)
- Pull Backs against elastic resistance (seated position)
- External shoulder rotation against elastic resistance (seated position)
- Steps on stairs (standing position)

2.11.2. Control exercise (flexibility exercise) program

Control exercise (flexibility exercise) program consisted of the following exercises:

- Hip and knee mobility (seated position)
- Hip mobility (standing position)
- Trunk and chest mobility (seated position)
- Shoulder mobility (seated position)
- Ankle mobility (standing position)

2.11.3. Program justification

SHEP was validated in the DO-HEALTH pilot trial [63] among 173 older adults with hip fracture. Results of the trial suggest that the program is successful in reducing the rate of falling by 25% (95% CI -44% to −1%) and the rate of fracture by 56% (95% CI −82% to 9%; $P = .08$) in the first year after the fracture [63]. Participants (65 years and older)

who performed the intervention at least once a week also had a significant improvement in lower extremity function (timed up-and-go) and reaction time (repeated chair stands) [63]. In DO-HEALTH, all participants were asked to develop a routine that allowed optimal adherence. This routine was documented by the study staff. Additionally, all participants were given a personal diary to record their adherence to the study intervention and the exercise program. The diary was collected at each clinical visit; it will not constitute a direct source of information for adherence, but it might be used pro re nata for confirmation of participant reported events.

2.11.4. Safety

Both exercise programs (SHEP and flexibility) tested in DO-HEALTH have been developed especially for the older population and entail only minimal risks and burden for the participants [63]. Both programs have already been tested in clinical study and shown to be well tolerated by seniors [63]. A possible side effect of the SHEP could be sore muscles after the first few training sessions. Since an experienced study nurse or movement scientist instructed the SHEP, the risk of other side effects is minimal.

2.12. Trial eligibility: inclusion and exclusion criteria

Inclusion and exclusion criteria are described in Table 1. Briefly, DO-HEALTH participants had to be ≥70 years, with a MMSE ≥ 24, living in the community, and sufficiently mobile to reach the study center. At the screening visit, participants were asked to take a placebo capsule to test whether they were able to swallow the study capsules.

2.13. Washout period

Volunteers meeting eligibility criteria except by taking 1000 IU or more of vitamin D per day in the 3 months prior to enrollment were subject to the following provisions:

- Provision 1: An individual who consumed an average vitamin D dose between 1000 and 2000 IU vitamin D/day in the 3 months prior to enrollment, could be enrolled after a 3-month wash-out period in which the maximum daily intake of vitamin D was limited to 800 IU.
- Provision 2: An individual who consumed an average vitamin D dose higher than 2000 IU/day in the 3 months prior to enrollment, could be enrolled after a 6-month wash-out period in which the maximum daily intake of vitamin D was limited to 800 IU.

2.14. Recruitment and randomization

2.14.1. Source of participants

DO-HEALTH participants were recruited through mailing lists of retirement authorities, churches, and other community services, posters, flyers, public events, advertisement in newspapers and other media, public events and educational programs and health care. The advertisements contained a contact phone number for each specific recruitment site that potential participants were asked to call for further information (DO-HEALTH telephone hotline – established at each recruitment site).

2.14.2. Randomization

Potentially eligible participants were randomized if serum calcium was below 2.6 mmol/L, creatinine clearance was above 15 mL/min (calculated according to the Cockcroft-Gault formula [85]), and the study medical doctor saw the participant and confirmed all inclusion and exclusion criteria.

Participants were randomized into the trial during the baseline visit. Randomization was computer-based (DO-HEALTH randomization software). Stratified block randomization was used (block size of 16 individuals). Willing and eligible participants were randomized to one of the eight treatment groups stratified by the study center, low trauma fall during previous 12 months prior to the randomization day (yes/no), age (70–84 and 85+), and sex. Within each group, treatment assignments were generated in blocks of sixteen individuals, with two individuals in each of the eight treatment combinations. Each study center was forced to keep a minimum balance between participants without falls and

Table 1
Inclusion/exclusion criteria.

Inclusion criteria	Exclusion criteria
1. Age ≥ 70 years 2. MMSE ≥24 3. Living in the community 4. Sufficiently mobile to reach the study center 5. Able to walk 10 m with or without a walking aid and getting in and out of a chair without help 6. Able to swallow study capsules 7. Able and willing to participate, sign informed consent (including consent to analyze all samples until drop-out or withdrawal) and cooperate with study procedures	1. Consumption of more than 1000 IU vitamin D/day in the 3 months prior to enrollment, or unwillingness to limit vitamin D intake to the current standard of 800 IU/day of vitamin D during the course of the trial (see washout period). 2. Unwillingness to limit calcium supplement dose to 500 mg/day for the duration of the trial 3. Taking n-3 s supplements in the 3 months prior to recruitment and or unwilling to refrain from use of n-3 s supplements for the duration of the trial 4. Use of any active vitamin D metabolite (i.e. Rocaltrol, alphacalcidiol), PTH treatment (i.e. Teriparatide), or Calcitonin at baseline and unwillingness to forego these treatments during the course of the trial 5. Current or recent (previous 4 months) participation in another clinical trial, or plans of such participation in the next 3 years (corresponding to DO-HEALTH length) 6. Presence of the following diagnosed health conditions in the last 5 years: history of cancer (except non-melanoma skin cancer); myocardial infarction, stroke, transient ischemic attack, angina pectoris, or coronary artery intervention 7. Severe renal impairment (creatinine clearance ≤15 ml/min) or dialysis, hypercalcaemia (> 2.6 mmol/l) 8. Hemiplegia or other severe gait impairment 9. History of hypo- or primary hyperparathyroidism 10. Severe liver disease 11. History of granulomatous diseases (i.e. tuberculosis, sarcoidosis) 12. Major visual or hearing impairment or other serious illness that would preclude participation 13. Living with a partner who is enrolled in DO-HEALTH (i.e. only one person per household can be enrolled) 14. Living in assisted living situations or a nursing home 15. Temporary exclusion: acute fracture in the last 6 weeks 16. Epilepsy and/or use of anti-epileptic drugs 17. Individuals who fell more than 3 times in the last month 18. Osteodystrophia deformans (M. Paget, Paget's disease) 19. For study center in Germany only: persons who were institutionalized / in prison by court order (§40, Abs. 1, Art. 4, "Gesetz über den Verkehr mit Arzneimitteln")

Legend: MMSE: Mini Mental State Examination; PTH: Parathyroid hormone.

participants with at least one fall in the last year prior to enrollment, with at least 40% of fallers. The centers were also required to monitor balance in the age and sex strata.

2.15. Blinding: study interventions and blood samples

2.15.1. Study capsules and exercise kits

Study capsules and exercise kits were blinded by a central randomization center. The producer of the study capsules (DSM Nutritional Products Ltd., Switzerland) sent the study capsule bottles, each one containing the monthly capsule supply, to the randomization center, which packaged the capsules together into kits of 12 bottles each (a one-year supply for one participant), labeling each bottle and the kit's box itself with a unique randomization code. Similarly, the exercise kits (video plus matching paper version) were labeled by the randomization center with another unique randomization code. The web-based randomization service, developed and supported by the DO-HEALTH software partner (FDS), stored the information linking the randomization codes to the actual content of the study capsule kits and exercise kits. No cases of unblinding was necessary, therefore there was no access to this code until the data set was frozen and the study was unblinded.

Kits of study capsules and exercise kits were sent to the recruitment centers with the individual coding. Externally, study capsule kits (vitamin D alone, n-3 s alone, combination, placebo) and exercise kits (strength or flexibility) were identical in appearance, except for the randomization code on the labels. All study staff members at each trial site were blinded to treatments, with the exception of the "instruction physiotherapist" who instructed the program at baseline (unblinding only with respect to the type of exercise) and was not part of the DO-HEALTH staff team. Thus, study interventions were double-blinded in DO-HEALTH, including the exercise program as participants were told that they would get one of two exercises.

2.15.2. Blood samples

The laboratory analyses and biobank storage were coordinated by the DO-HEALTH Data Management Team with restricted access to results to avoid potential un-blinding via laboratory results. The handling and labeling of all samples were anonymous and standardized, and implemented by the coordinating team of DO-HEALTH at the coordinating site in Zurich.

2.16. Adherence assessment

2.16.1. Study capsules adherence

Adherence to study intervention was assessed by blood levels of 25 (OH)D and polyunsaturated fatty acids (PUFA) in all participants at 12, 24, and 36 months. DSM Analytical Research Center performed 25(OH) D serum measurements with gold standard HPLCMS/MS methodology, and PUFA measurements by a sensitive and selective assay based on gas chromatography coupled to mass spectrometry detection (GC–MS). DSM Analytical Research Center is accredited according to ISO 9001:2008.

Adherence was also monitored at each contact (3-monthly phone calls and clinical visits at 12, 24, and 36 months) by participant self-report, at the same time investigating the reasons why or why not participants adhered to the study intervention and/or any difficulties encountered in following the intervention regimen. Finally, participants were asked to bring all used, partially used, and full bottles of study capsules to the clinical visits at 12, 24, and 36 months for pill counts.

2.16.2. Study exercises adherence

Adherence to the exercise program was monitored at each contact (3-montly phone calls and clinical visits at 12, 24, and 36 months), by participant self-report. We also investigated the reasons why or why not participants adhered to the exercise program and/or any difficulties encountered in following the program without asking about which exercise program was being used by the participant. If any of the exercises caused problems, the assessment staff asked the "instruction physiotherapist", who was unblinded to the type of exercise, to contact the participant. Adherence was supported by motivational strategies at each contact (every 3 months).

2.16.3. Safety monitoring

At each clinical visit, safety markers (calcium and creatinine clearance) were analyzed by express tests performed at the laboratories of each trial site. Overall, the adverse events with vitamin D and n-3 s were expected to be rare [25,37,63,86]. Also, the home exercise program was well tolerated in the pilot trial among hip fracture patients, and adverse events were also expected to be rare [63].

2.16.4. Adverse events definition

Participants were questioned about adverse events (AEs) at each study visit and at each 3-monthly phone contact. AEs or abnormal test findings potentially associated with the study treatment(s) were followed until the event (or its sequelae) or the abnormal test finding resolved or stabilized.

For each AE, the investigator was provided with the onset, duration, intensity, treatment required, outcome and action taken with the investigational product.

The intensity of AEs was assessed as being: mild (hardly noticeable, negligible impairment of well-being), moderate (marked discomfort, but tolerable without immediate relief), or severe (overwhelming discomfort, calling for immediate relief).

2.16.5. Serious adverse events definition

Serious adverse event (SAEs) were defined as any untoward medical occurrence that at any dose: resulted in death; was life-threatening; required subject hospitalization or prolongation of current hospitalization; resulted in persistent or significant disability/incapacity; or any important medical event and any event which, though not included in the above, may jeopardise the subject or may require intervention to prevent one of the outcomes listed above. All SAEs were reported to the sponsor of the trial. The Data Safety and Monitoring Board (DSMB) consisted of 3 independent experts and without any other relationship to the DO-HEALTH study, no financial interest in the outcome of the study, and was excluded from authorship of study findings. The DSMB examined the progress and safety data of the DO-HEALTH trial and produced an annual statement to recommend continuation of the trial.

2.16.6. Analysis plan and statistical power

All analyses plans were pre-defined and reviewed by the PI, the DO-HEALTH epidemiology team, and the head biostatistician. No interim analysis was planned, and none was requested by the Data Safety and Monitoring Board. All necessary programs for the analyses and for the preparation of datasets were coded in the SAS statistical language and stored as electronic SAS files in the designated storage folders.

The epidemiology team at the DO-HEALTH coordinating center will conduct all statistical analyses using SAS v9.4 statistical software (Copyright© 2004 by SAS Institute Inc., Cary, NC, USA). To account for multiple comparisons, the significance threshold will be set at $p < .01$ for the primary outcomes. In analyses for all other endpoints, a p-value of 0.05 (two-sided) will be considered a threshold for statistical significance.

2.16.7. Analysis of treatment effects

In this $2 \times 2 \times 2$ factorial design, the primary aim is to compare the main effects and the combined effects of vitamin D, n-3 s, and the SHEP on the 6 primary endpoints. All analyses will be performed based on intent-to-treat, so that all available data will be included for all study participants.

Our general approach is to use longitudinal linear regression models for continuous outcomes (systolic and diastolic blood pressure and MoCa), with changes from baseline at 1, 2, and 3 years as outcomes,

adjusting for correlation between serial measurements from the same patient. For the non-normally distributed outcomes (SPPB), Generalized Estimating Equations (GEE) with an unstructured correlation matrix will be used to account for correlation within participants over time. These models will include fixed effects for the interventions, as well as adjust for the stratification variables (study center, age, sex, prior fall), the appropriate baseline outcome measure, time as a categorical predictor, intervention by time interaction effects (if significant), and baseline BMI as a pre-defined potential confounder. For non-vertebral fractures and infections, we will use the analogous Poisson regression model with each participant's time in the study as an exposure offset.

First, these models will be used to determine for each outcome whether the intervention effects are additive. In addition to the main intervention effects and the predictors described above, we will include two- and three-way interaction terms between interventions. For outcomes for which interactions are found, all subsequent models will treat the study as an 8-armed trial with indicator variables for the 8 intervention combinations. For outcomes for which interactions are not significant, all subsequent models will include only the main effect of each intervention (plus adjustment covariates); combined effects of the interventions will be estimated by adding the individual effects.

Second, these models will be used to determine the longitudinal effects of the interventions across all 3 years of follow-up. For outcomes with no treatment interactions, the group of patients who received an intervention is compared to the group who did not. For outcomes with treatment interactions, the arm that received a particular intervention along with no other interventions was compared to the arm that received none of the three interventions. For each outcome, the initial model will include indicators for the intervention groups, indicators for time, and interactions between interventions and time to assess whether there are progressive benefits due to intervention over time (adjustments for covariates and correlation remain as described above). If interaction terms are found to be non-significant, they will be removed, leaving the main effect of each intervention to represent the consistent, average impact of the intervention across all three years. See Appendix 2 for model equations.

2.16.8. Power

The planned sample size for this study was $N = 2152$ older adults (Table 2). Based on prior experience and results of pilot studies the dropout rate was estimated at 32%, while 68% of participants were expected to complete the entire 36-month follow-up. Since the trial analysis is based on the intention-to-treat principle, partial study data would be available from the 32% of subjects who are expected to drop out early. Therefore, the estimated effective sample size for the analysis was 1807 people: full follow-up data on 68% of the 2152 enrolled older adults ($0.68 \times 2152 = 1463$), plus an average of half follow-up on the 689 of subjects who were expected to withdraw early ($0.5 \times 0.32 \times 2152 = 344$).

2.17. Missing data

Handling of missing data for the 6 primary outcomes depends on the amount of data being missing. If <1% of data are missing, it will be considered as missing completely at random (MCAR)and we will perform complete-case analysis, which yields unbiased parameter estimates albeit with reduced statistical power.

For continuous primary outcomes (function; blood pressure; and cognition), if >1% of data are missing, analysis will be performed using models assuming data are missing at random such as the mixed effects model. Sensitivity analysis will also be performed using multiple imputation, which is implemented in SAS via the 'PROC MI' and 'PROC MIANALYZE' procedures.

For binary (non-vertebral fractures) and ordinal (number of infections) outcomes, statistical analysis models will adjust for time-in-study and no imputation will be performed.

Handling of missing data for secondary outcomes follow the same procedure described above for the primary outcomes.

2.18. Ethics

DO-HEALTH was approved by local/national ethics committee and regulatory authorities of all 5 countries (Switzerland, Germany, Austria, France, Portugal). DO-HEALTH was a Phase III trial (therapeutic confirmatory) and was conducted according to the ICH GCP Guidelines

Table 2
Power considerations for the primary endpoints.[a]

Endpoint	Minimum detectable difference[b]	Standard Deviation	Alpha	Power with no interaction, $N = 1806$ (903 vs 903)[c]	Power with interaction, $N = 902$ (451 vs 451)[d]
Cardiovascular[e]					
Systolic blood pressure (mean, mmHg)	6	24.5	0.01	0.99	0.82
Diastolic blood pressure (mean, mmHg)	3	12.4	0.01	0.99	0.86
Bone[f]	0.073[g]	–	0.01	0.99	0.80
Incidence rate of any non-vertebral fracture including hip fractures (proportion)					
Muscle[e]	0.40	1.41	0.01	0.99	0.97
Lower extremity function (mean SPPB score)					
Brain[e]	0.7	2.3	0.01	0.99	0.90
Cognitive function (mean MoCA score)					
Immunity[h]	0.32	$s_1 = 1.46$	0.01	0.99	0.90
Incidence rate of any infection		$s_2 = 1.35$			

[a] All assumptions about means and standard deviations of the parameters were based on the results of pilot trials to DO-HEALTH [37,63,87] and research of other authors [88–90].

[b] Minimum detectable difference was assumed as a minimum difference that is clinically significant.

[c] Power assuming 32% of lost to follow-up and no statistical interactions between the three treatment groups. Power with no interaction was calculated in case we found no treatment-by-treatment interactions; then half of the total sample who received a particular intervention could be compared to the other half who did not receive that intervention.

[d] Power assuming 32% of lost to follow-up and statistical interactions between the three treatment groups. Power with interaction was calculated in case we found a treatment-by-treatment interaction; then only the one-eighth of the total sample who received a particular intervention alone could be compared to the one-eighth of the total sample who received no active intervention.

[e] Power calculations were based on the two-sample *t*-test assuming equal variances.

[f] Power calculations were based on the *Z*-test for difference between two proportions.

[g] Estimated baseline prevalence of any non-vertebral fractures incidence rate / IR = 0.14.

[h] Power calculations were based on Poisson regression assuming non-equal variances.

(CPMP/ICH/135/95).

2.19. Trial registration and funding

DO-HEALTH is registered under the protocol NCT01745263 at the International Trials Registry (clinicaltrials.gov), and under the protocol number 2012–001249-41 at the Registration at the European Community Clinical Trial System (EudraCT).

DO-HEALTH was funded by the European Commission Framework 7 Research Program (project number 278588), the University of Zurich (Chair of Geriatrics and Aging Research), and independent, investigator initiated funds provided by DSM Nutritional Products, ROCHE Diagnostics (SCHWEIZ), NESTEC, Pfizer Consumer Healthcare, and STREULI Pharma. The funders had no role in the DO-HEALTH study design or writing of the report.

3. Discussion

DO-HEALTH is the largest European healthy aging trial including 5 European countries. The goal of the DO-HEALTH trial was to address 6 primary endpoints (systolic and diastolic blood pressure, non-vertebral fractures, SPPB [34], MoCA [35], and infections) relevant to healthy aging among community-dwelling adults age 70 years and older. In this framework, DO-HEALTH was designed, through intensive monitoring of 2152 participants every 3 months, to address changes in important risk factors, such as functional/cognitive decline and elevated blood pressure, rather than major clinical events such as dementia, myocardial infarction or stroke. The 3 interventions tested in DO-HEALTH: n-3 s, vitamin D, a simple home strength exercise, were selected to be well-tolerated and affordable, allowing, if proven effective, to be implemented at a large-scale public health level.

DO-HEALTH has several strengths. It targets the relatively healthy 70+ age segment of the population expected to grow rapidly in the coming years, at an age where disease rates are increasing exponentially. Further, as a healthy aging trial, it is powered for 6 primary endpoints from 5 health domains: systolic and diastolic blood pressure changes (cardiovascular), incidence of non-vertebral fractures (bone), functional decline (muscle), cognitive decline (brain), and rate of infection (immunity). Additionally, a series of pre-defined and well-integrated secondary endpoints and ancillary studies were designed to explore the role of the interventions also with regard to joint, mental, dental, gastro-intestinal, glucose-metabolic, kidney and global health. Its detailed follow-up including yearly whole-day clinical visits and 3-monthly phone calls in all participants includes the assessment of key life-style factors such as nutrition, education, and physical activity, as well as comorbidities and the use of prescription drugs and over the counter supplements. The factorial design (2×2×2) was chosen to identify the role of the individual and combined effects of the 3 interventions. Blood collection and analysis of 25(OH) D and PUFAs (EPA + DHA), were assessed in all participants at baseline, 12, 24, and 36 month follow-up and thereby provide an excellent measure of adherence to the study medication. Further, the extensive 55-item DO-HEALTH biomarker study (Supplementary Table 1), assessed in all participants at all time points, support the DO-HEALTH clinical endpoints at the mechanistic level and will help extend our understanding of the effect of the interventions in relation to many well-integrated additional secondary and tertiary endpoints to be explored in pre-defined ancillary studies.

Finally, because DO-HEALTH was a multicenter study with detailed and standardized phenotyping of all participants, health disparities between the 5 European countries will be also explored, as will the genetic profiles depending on further funding availability for the biobank of DO-HEALTH.

Our trial also has limitations. Only one dose of vitamin D and n-3 s was tested. However, the dose for each agent was chosen based on an extensive review of available evidence, including several pilot studies [37,63] and meta-analyses [25,93,94]. Further, serum baseline and achieved concentration of the related biomarkers 25(OH)D and EPA + DHA will be analyzed to test changes in the endpoints addressed in DO-HEALTH by changes in the biomarkers. Another limitation may be that in order to have a placebo group in DO-HEALTH, we allow all participants to take 800 IU vitamin D per day according to current guidelines. This may bias our findings for vitamin D towards a conservative comparison between the tested higher dose of 2000 IU to 800 IU. Finally, our findings may not be applicable to younger adults, or less healthy community-dwelling adults age 70 years, or older or institutionalized older adults.

Given the broad popularity of supplement use of vitamin D and n-3 s, the clarification of their role in supporting several endpoints among the growing segment of older adults is of enormous public health relevance. For vitamin D, the evidence for the prevention of falls and fractures, especially among healthy older adults without osteoporosis and vitamin D deficiency have been broadly discussed [6,95–98] and needs further clarification from a large clinical trial.

Marine n-3 s have shown considerable promise for the secondary prevention of cardiovascular disease in high-risk settings [99–101]. Few studies, however, addressed the long-term use of n-3 s in primary prevention of cardiovascular-health. Further, current data from clinical trials are limited and inconsistent regarding the role of n-3 s with regard to bone, muscle, brain, and immunity [102–109]. DO-HEALTH will advance the state of the science by comparing the long-term effect of n-3 s against placebo and combined with vitamin D and/or exercise.

Physical exercise has shown considerable promise as a comprehensive prevention strategy for many age-related diseases, such as osteoporosis [110], and fall prevention [111–115], as well as cardio-vascular health [32,116–118]. However, data on fracture reduction are lacking from randomized controlled trials, and the role of a well-defined simple home exercise program with regard to several endpoints relevant to aging, as tested in DO-HEALTH, needs further clarification.

In conclusion, we expect that DO-HEALTH findings will have a large impact on public health as the interventions can be easily implemented in clinical practice, and the endpoints are relevant to the overall health of older adults because they address several health domains including cardiovascular, muscle, bone, brain and immunity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cct.2020.106124.

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