Study Protocol

Study Title

Vitamin D3 – Omega3 – Home Exercise – HeALTHy Ageing and Longevity Trial

Randomized, double-blind, placebo-controlled, multi-centre clinical trial

Acronym: DO – HEALTH

Date **March 3rd 2015**

Version n° **3.0**

EudraCT n° **2012-001249-41**
Protocol n“ **KEK-ZH-2012-0249**

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Key sections at a glance

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2. Abbreviations

25(OH)D – 25-hydroxyvitamin D
AE – Adverse Event
AHRQ – Agency for Healthcare Research and Quality
CHARITE – Charité Clinic Berlin
CHUT - University Hospital Center Toulouse
CPM – Clinical Project Manager
CRA – Clinical Research Administrator
CRF – Case Record Form
CRP – C-reactive Protein
CVD – Cardiovascular Disease
DASH - Disabilities of the Arm, Shoulder and Hand
DHA – Docosahexaenoic Acid
DSMB – Data Safety and Monitoring Board
EDC – Electronic Data Collection
EFGR – Epidermal Growth Factor Receptor
EPA – Eicosapentaenoic Acid
FDS – Ferrari Data Solutions
GCP – Good Clinical Practice
GDS – Geriatric Depression scale
GOHAI – Geriatric Oral Health Assessment Index
HOOS – Hip injury and Osteoarthritis Outcome Score
IGF-1 – Insulin-like Growth Factor-1
IL– Interleukin
IL-8 – Interleukin 8
IL-10 – Interleukin 10
IMU – Innsbruck Medical University
IOF – International Osteoporosis Foundation
IOM – Institute of Medicine
IPC – Intellectual Property Committee
IPR – Intellectual Property Rights
KOOS – Knee injury and Osteoarthritis Outcome Score
MI – Myocardial Infarction
MMSE – Mini Mental State Examination
MoCA – Montreal Cognitive Assessment
MRI – Magnetic Resonance Imaging
NHS – Nurses' Health Study
NSAE – Non-serious Adverse Event
OA – Osteoarthritis
OR – Odds Ratio
PROMIS-HAQ – Patient-Reported Outcomes Measurement Information – Health Assessment Questionnaire
PUFA – Polyunsaturated Fatty Acids
RAAS – Renin-Angiotensin-Aldosterone System

RCT – Randomized Clinical Trial
RR – Relative Risk
SAE – Serious Adverse Event
SHARE-FI – Survey of Health, Ageing and Retirement in Europe Frailty Instrument
SME – Small and Medium Enterprise
SOP – Standard Operating Procedure
SPPB – Short Physical Performance Battery
TNF-α – Tumor Necrosis Factor α
UZH – University of Zurich
VDR – Vitamin D Receptor
VEGF – Vascular Endothelial Growth Factor

3. Abstract

As the European population is ageing rapidly, the growing number of seniors with age-related chronic diseases poses a challenge on European societies and health care systems. Therapeutic interventions that are effective, affordable, and well-tolerated in the prevention of chronic disease are urgently needed and will have an outstanding impact on public health as a whole. Among the most promising interventions that meet these requirements are vitamin D, marine omega-3 fatty acids and physical exercise. However, their individual and combined effects have yet to be confirmed in a clinical trial. DO-HEALTH will close this knowledge gap in a large 3-year multi-centre clinical trial that will establish long-term efficacy and safety data for the 3 interventions in the prevention of age-related diseases in seniors.

DO-HEALTH will enroll 2152 community-dwelling men and women who are 70 years and older, an age when chronic diseases increase substantially. DO-HEALTH will test the individual and the combined benefit of 2000 IU vitamin D/day, 1 g of omega-3 fatty acids/day and a simple home exercise program in an efficient randomized-controlled factorial design trial. The trial will define the role of the 3 interventions in the prevention of 5 primary endpoints: the risk of incident non-vertebral fractures; the risk of functional decline; the risk of blood pressure increase; the risk of cognitive decline; and the rate of any infection. Key secondary endpoints include risk of hip fracture, rate of falls, pain in symptomatic knee osteoarthritis, gastro-intestinal symptoms, mental and oral health, quality of life, and mortality. Follow-up will be in-person, in 3-monthly intervals (4 clinical visits and 9 phone calls). DO-HEALTH will further assess the comparative effectiveness of the interventions by evaluating reasons why or why not seniors adhere to them, and will assess their cost-benefit in a health economic model based on documented health care utilization and observed incidence of chronic disease.

4. Study Synopsis

Title	**Vitamin D3 – Omega3 – Home Exercise – HeALTHy Ageing and Longevity Trial** Acronym: DO-HEALTH
Sponsor & Coordinator & Principal Investigator	Prof. Heike A. Bischoff-Ferrari, MD, DrPH Centre on Aging and Mobility, Zurich, Switzerland
Protocol version, Date	**Version 3.0, March 3, 2015**
Objectives	**Main objectives:** • To improve healthy ageing in European seniors • To reduce healthcare costs via the implementation of effective and broadly applicable disease prevention interventions **Specific Objectives:** • To establish whether vitamin D, omega-3 fatty acids, and a simple home exercise program will prevent disease at older age • To assess comparative effectiveness and cost-benefit of the interventions
Participants / Study population justification	2152 seniors will be recruited; of these, at most 1291 (60%) will be apparently healthy (see inclusion criteria), and at least 861 (40%) will be pre-frail (low trauma fall event) community-dwelling seniors. *Study population justification:* DO-HEALTH will enroll seniors age 70 years and older for their high risk of chronic disease, their high risk of vitamin D and omega-3 deficiency, and their high prevalence of physical inactivity. To represent the largest part of the senior population, DO-HEALTH will recruit community-dwelling seniors. However, to represent also the pre-frail population at risk of institutionalization, at least 40% of seniors will be enrolled based on a low trauma fall with or without a fracture in the year before DO-HEALTH enrollment
Trial design	Under the assumption that vitamin D, omega-3 fatty acids, and the home exercise program have distinct mechanisms of action and therefore will have an additive effect on the study endpoints, we chose a **2×2×2 factorial study design as being the most efficient**. All analyses will test the effect of each strategy, while controlling for the others
Trial Interventions	There will be 8 treatment arms – see allocation of treatment groups (Table 1, Page 49) **The 3 primary treatment comparisons are:** (1) dietary supplement of 2000 IU vitamin D per day compared to placebo (controlling for the other treatment strategies) (2) dietary supplement of 1 gram of omega-3 fatty acids (EPA+DHA, ratio 1:2, from marine algae) compared to placebo (controlling for

	the other treatment strategies) (3) Home exercise program (Strength) of 30 minutes 3 times a week compared to a control exercise program (Flexibility) 30 minutes 3 times a week
Primary Endpoints	**The DO-HEALTH trial will address 5 primary endpoints:** (1) *Bone*: Incident non-vertebral fractures over 36 months (confirmed by X-ray or medical reports) (2) *Muscle*: Functional decline (assessed by Short Physical Performance Test Battery at baseline, 12, 24 and 36 months) (3) *Cardiovascular*: Systolic and diastolic blood pressure change (at baseline, 12, 24 and 36 months) (4) *Brain*: Cognitive decline (assessed by Montreal Cognitive Assessment at baseline, 12, 24 and 36 months) (5) *Immunity*: rate of any infection (at baseline, and every 3 months up to 36 months) *A Bonferroni adjustment for multiple comparisons will apply so that p<0.01 is required for significance*
Secondary Endpoints	**The DO-HEALTH trial will assess additional secondary endpoints that support the primary endpoints and extend to other organ systems:** (1) *Bone:* incidence of hip fractures (at 36 months), incidence of new vertebral fractures (vertebral morphometry in a subset of 1502 seniors with yearly DXA measurements; at 36 months), incidence of total fractures – (combined non-vertebral + new vertebral fractures in subset of 1502 seniors with yearly DXA measurements; at 36 months), risk of bone mineral density decrease in the spine and hip (in subset of 1502 seniors with yearly DXA measurements; at baseline, 12, 24, 36 months) (2) *Muscle:* rate of falling (rate of any low trauma fall, rate of injurious falls, number of persons who fell; assessed every 3 months over 36 months), reaction time and grip strength (at baseline, 12, 24, 36 months), incidence of muscle mass decrease at the upper and lower extremities (in a subset of 1502 seniors with yearly DXA measurements at baseline, 12, 24, 36 months), musculoskeletal pain (at baseline, 12, 24, 36 months), dual tasking 10 meter gait speed (at baseline, 12, 24, 36 months) (3) *Cardiovascular:* risk of incident hypertension (at 36 months) (4) *Brain:* mental health decline, incident depression (at baseline, 12, 24, 36 months), dual tasking gait variability in a subset of 250 participants (at baseline, 12, 24, 36 months) (5) *Immunity:* rate of any upper respiratory infection, incident flu-like illness, incident severe infections that lead to hospital admission (assessed every 3 months over 36 months) (6) *Bone/Cartilage: Arthritis:* primary outcome for osteoarthritis will be severity of knee pain (KOOS) in those with symptomatic knee

	osteoarthritis (based on modified clinical ACR criteria), secondary outcomes for osteoarthritis will be: rate of knee buckling, NSAID use because of knee pain; number of joints with pain (all cartilage endpoints are assessed at baseline, 12, 24, 36 months) (7) *Dental:* decline in oral health, tooth loss (at baseline, 12, 24, 36 months) (8) *Gastro-intestinal:* gastro-intestinal symptoms based on Rome III questionnaire (at baseline, 12, 24, 36 months) (9) *Glucose-metabolic:* fasting glucose and insulin levels, QUICKI and HOMA index, body composition and increase in body fat in the trunk and extremities by DXA (in subset of 1502 seniors with yearly DXA measurements; at baseline, 12, 24, 36 months) (10) *Kidney:* decline in kidney function – by blood creatinine levels and estimated glomerular filtration rate (at baseline, 12, 24, 36 months) (11) *Global Health:* quality of life (assessed every 6 months), incident frailty, incident disability regarding activities of daily living (at baseline, 12, 24, 36 months), incident nursing home admissions, rate of acute hospital admissions, mortality (assessed every 3 months)
Biomarker Endpoints	The DO-HEALTH trial will include **an organ-specific biomarker study** to **support primary and secondary endpoints at a mechanistic level** (assessed in 2152 seniors at baseline, 12, 24, 36 months of follow-up): (1) *Bone:* calcium, phosphate, 25(OH)D, intact PTH, urinary calcium/creatinine ratio (second spot urine), Beta-Crosslaps serum, P1NP (2) *Cardiovascular:* Troponin T, NT-proBNP, homocysteine, CK, cholesterol, HDL-cholesterol, triglycerides (3) *Inflammation:* hs-CRP, IL6 (4) *Gastrointestinal:* AST, ALT, gGT, alkaline phosphatase, bilirubin (5) *Glucose-metabolic:* fasting glucose, insulin (6) *Kidney:* serum creatinine; calcium/creatinine ratio in second spot urine, serum urea, uric acid (7) *Global Health: Ions*: sodium, potassium, chloride, magnesium; *Proteins*: total protein, albumin, ferritin, transferrin; *Hormones*: TSH, fT4, fT3, cortisol; *vitamins*: folic acid, vitamin B12, 25(OH)D **Biomarker study for novel bone and muscle functionality:** assessed at baseline, 12, 24, 36 months in 2152 seniors: (1) *Bone:* sclerostin; (2) *Muscle:* myostatin **Biomarker study for novel inflammation and immunity markers:** assessed at baseline, 12, 24, 36 months in the 802 seniors recruited by 2 centers (Zurich and Basel) (1) *Inflammation:* TNF-α, IL-10, IL-17, IL-22

	(2) *Cellular immunity:* CD3, CD4, CD25, CD127
Exploratory Endpoints	The DO-HEALTH trial will assess exploratory endpoints that support the primary endpoints, but have limited statistical power (assessed over 36-month follow-up): (1) *Bone:* Incident repeat fractures (any repeat non-vertebral fractures in all participants, vertebral fractures and total fractures among subset of 1502 seniors with yearly DXA measurements) Ancillary fracture healing study in all seniors with an incident major osteoporotic fractures at the arm (shoulder, humerus, forearm) and leg (hip, femur, ankle): (a) *primary fracture healing endpoint*: clinical fracture healing with 3 additional phone calls at 6, 12, 18 weeks after the fracture by PROMIS-HAQ questionnaire (b) *secondary fracture healing endpoint*: observed functional fracture healing measured with the Short Physical Performance Test Battery and grip strength at regular 12, 24, 36 month visits (c) *exploratory fracture healing endpoint*: radiological fracture healing with independent assessment of early (6-8 weeks) and late (12 to 14 weeks) consolidation assessment based on standard clinical care X-rays – participants will report a new fracture 7 days of the event (recruitment centre hotline) (2) *Muscle:* incident sarcopenia (among subset of 1502 seniors with yearly DXA measurements), incident frailty, decline in physical activity (at 36 months) (3) *Cardiovascular:* major cardiovascular events as a composite endpoint (any event: myocardial infarction, stroke, revascularization procedures of CABG and PCI, incident congestive heart disease, cardiovascular mortality); individual endpoints: myocardial infarction, stroke, incident congestive heart disease, and cardiovascular mortality (assessed every 3 months over 36 months) (4) *Brain:* incident dementia (at 36 months) (5) *Immunity:* incident cancer (any cancer, gastro-intestinal, breast cancer in women, prostate cancer in men); rate of implant infections after total hip or knee replacement (due to fracture or osteoarthritis); rate of gastro-intestinal infections (at 36 months) (6) *Bone/Cartilage-Arthritis:* incident symptomatic knee osteoarthritis; incident symptomatic hip osteoarthritis, incident symptomatic hand osteoarthritis; composite endpoint: incident symptomatic knee, hip or hand osteoarthritis; severity of hip pain in those with prevalent symptomatic hip osteoarthritis, severity of hand pain in those with prevalent symptomatic hand osteoarthritis (assessed every 12 months over 36 months)
Adherence Laboratory and Safety Endpoints	**Adherence laboratory** (assessed at baseline, 12, 24, 36 months follow-up in 2152 seniors): *serum 25(OH)D* concentrations (measured both by an automated assay and HPLCMS/MS) and *plasma PUFA concentrations* (EPA, AA, DPA, DHA; measured by a sensitive and selective assay based on gas chromatography coupled to mass spectrometry detection (GC-MS)).

	Safety laboratory (assessed at baseline, 12, 24, 36 months follow-up in 2152 seniors)**:** serum calcium and serum creatinine
Inclusion Criteria	(1) Age 70 years or older (2) Mini Mental State Examination Score of at least 24 (3) Living in the community (4) Sufficiently mobile to come to the study centre (5) Able to walk 10 meters with or without a walking aid and able to get 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
Exclusion Criteria	(1) Consumption of more than 1000 IU vitamin D/day in the 6 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 i. 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, may be enrolled after a 3-month wash-out period where the maximum daily intake is limited to 800 IU vitamin D. ii. Provision 2: an individual who consumed an average vitamin D dose higher than 2000 IU/day in the 3 months prior to enrollment, may be enrolled after a 6-month wash-out period where the maximum daily intake is limited to 800 IU vitamin D. (2) Unwillingness to limit calcium supplement dose to 500 mg per day for the duration of the trial (3) Taking omega-3 fat supplements in the 3 months prior to recruitment and or unwilling to refrain from use of omega-3 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. tubercolosis, 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 are institutionalized / in prison by court order (§40, Abs. 1, Art. 4, "Gesetz über den Verkehr mit Arzneimitteln")
Sample Size	2152 participants (269 in each of the 8 treatment groups). See study schema page 39 (Figure 3) for detailed allocation of treatment groups
Power	The sample size of 2152 senior participants was chosen to have sufficient power for the most critical primary and secondary endpoints (any non-vertebral fracture and hip fracture) and, based on prior experience, the expectation that 68% will complete the entire 3-year study. However, the subjects who drop out early will provide partial data since our analyses will be based on the intention-to-treat principle. Under these assumptions and assuming **an additive effect of the 3 interventions,** as expected from our pilot study (7), we have **> 90% power for all 5 primary endpoints** (with Bonferroni adjustment for multiple comparisons). On the other side, **if an interaction between interventions is assumed, the power still exceeds 80%.** Contributing to the statistical power for the most critical fracture endpoints is the fact that at least 40% of seniors will be recruited based on a prior fall with or without a fracture, which confers an increased risk of sustaining a fracture endpoint during the course of the trial
Randomization	**Stratified Block Randomization** with block sizes of 16 individuals (2 for each of the 8 treatment combinations) will be performed by the DO-HEALTH randomization software. Labeling of study intervention will be performed by a central randomization centre in Switzerland. **Stratification variables:** recruitment centre (7 centers), low trauma fall during previous 12 months prior to the randomization day (yes/no), gender, and age (70 – 84 and 85+). The recruitment of at least 40% of participants who fell during the last year will be enforced at each of the 7 recruitment centers. Gender and age distribution will be monitored

	within each recruitment centre with the DO-HEALTH randomization software. If gross imbalance (less than 30% of participants in a stratum) is detected within a centre, recruitment strategies for the centre will be adapted to boost recruitment of participants of underrepresented category
Timeline	**Project Begins (preparation phase):** January 1st, 2012 **Screening phone calls**: start in December 2012 **Recruitment Period:** December 2012 – November 2014 **25% of Participants Recruited:** August 2013 **Baseline Clinical Visit Completed:** November 2014 **12-month Clinical Visit Completed:** November 2015 **24-month Clinical Visit Completed:** November 2016 **Follow-up Completed:** November 2017 **Project Completed:** May 2018
Recruitment strategies	Recruitment potential and feasibility of clinical visit burden has been established among the target population in a pilot trial of DO-HEALTH (Zurich Disability Prevention Trial: NCT01017354). DO-HEALTH will use mailing lists of retirement, church and other community services, posters, flyers, newspaper and other media advertisements, public events and education programs. Seniors in all recruitment countries were involved in testing the recruitment and information material to ensure that all information on the DO-HEALTH trial participation is understood
Recruitment goals	See table below DO-HEALTH has set a recruitment goal of 25% at each recruitment centre by month 20 (6 months after recruitment start in the last fully approved recruitment centre). **If a centre does not meet its recruitment goal by month 20 (end of August 2013) or is unable to recruit seniors with or without a fall in a balanced manner (with at least 40% of fallers), the centre will be closed for further recruitment and the recruitment load will be shifted to other centers that showed a recruitment rate beyond**

Recruitment Centre	Planned number of participants	Actual number of recruited participants
Zurich University Hospital, Switzerland	552	552
University of Geneva, Switzerland	200	201
Basel University Hospital, Switzerland	250	253
Toulouse University Hospital, France	300	301
Charité Clinic, Germany	350	350
Innsbruck Medical University, Austria	200	201
Coimbra University, Portugal	300	301
Total	**2152**	**2159**

	their estimate. Alternatively, if this is not possible, a new recruitment centre (at the University of Dresden) will be opened.
Follow-up	**Follow-up Period:** 3 years **Four Clinical Visits:** baseline, follow-up visits at 12, 24, and 36 months. Ideally, all visits should be completed in one day, however, a center is allowed to split the visits over two days to streamline the workflow, or prevent the participants from being overwhelmed. Two conditions need to be respected: • at baseline, the participant is randomized on the second day of the split visit • the second part of any visit (baseline, 12, 24, 36 month) should take place no later than 1 week from the first part **Nine Telephone Follow-up Interviews:** every 3 months between the clinical visits *Justification of in- person follow-up:* Clinical visits are at baseline, 12, 24 and 36 months in all 2152 participants, with additional 3-monthly contact by phone calls in all participants. Based on prior experience, we designed DO-HEALTH to have an age appropriate follow-up in 3-month intervals. This will allow for a high-quality endpoint assessment. Seniors tend to forget health events, which will be avoided in the best possible manner in our follow-up structure. This will be also supported by an event diary. Furthermore, based on prior experience, seniors tend to be overwhelmed by questionnaires sent to them by mail. We believe that the in-person follow-up will address that concern. *Justification of 3-year treatment*: Based on our pilot studies – pooled participant-level analysis of 11 RCTs (8) plus DO-HEALTH pilot trial (7), the 36 months of follow-up will provide enough participant-years to accumulate a sufficient number of events for the most critical primary (any non-vertebral fracture) and the most critical secondary endpoint (hip fracture), especially with respect to the 50% recruitment of pre-frail seniors with a prior fall/fracture event. Further, 3 years of follow-up will establish long-term efficacy and safety data for the 3 interventions.
Adherence Assessment/ **Assessment of comparative effectiveness**	Adherence to the interventions will be assessed at each clinical visit by: (1) Count of unused study capsules (2) Measurement of blood levels of 25(OH)D and PUFAs (3) 3-month in person adherence and comparative effectiveness assessed by a questionnaire (why and why not participants are adherent to the study interventions) (4) All participants will be given a personal diary to record their adherence to the study intervention and the exercise program. The diary will be collected at each clinical visit; it will not constitute a direct source of information for endpoint assessment, but might be used *pro re nata* for confirmation of participant reported events (5) Standardized motivation for the exercise program at 3-monthly in

	person contacts and reminder to take study capsules
Data Collection, Storage, and Quality Control	DO-HEALTH will use **specially designed electronic data collection system**. The software will be able to detect and flag data entry errors in real time. All data collected at the study centre will be securely transferred to the Central Data Bank at the end of each working day. Each study centre will have dedicated backup storage that will keep all data collected by the study centre. Central Data Bank will store the entire study database and will be equipped with a dedicated backup storage and additional secure off-site backup storage. Study database will be backed up daily. DO-HEALTH will employ specially designed data collection procedures facilitating accurate data collection and double-checking of critical data points before a clinical visit or a phone call is complete. Data collection procedures will be augmented by error detection software routines programmed into the electronic data collection system.
Blood collection and storage	Blood samples collected from the participants will be temporarily stored at each study centre at -80°C. Aliquots will be transferred to the central biobank on a 3-monthly basis in dry ice containers, so that the cold chain is not disrupted, and stored in the central DO-HEALTH biobank at Fisher Clinical Services GmbH (Allschwil, Switzerland) under the same temperature conditions until analyzed (for the DO-HEALTH biomarker study and later exploitation).
Statistical Analyses	All statistical analyses of DO-HEALTH participant data will be performed centrally at the coordinating DO-HEALTH centre at the University of Zurich by the DO-HEALTH data management team, based on pre-defined analysis plans and under supervision of the DO-HEALTH head biostatistician (Prof. E. Orav). All analyses for the DO-HEALTH trial will be performed based on the intent-to-treat principle after all study data are collected. Further, a per-protocol analysis is foreseen among adherent individuals (≥80% adherence). The statistical models will assess the effect of treatment, considering stratification variables, as well as pre-defined potential confounders. We will assess the effect of one treatment while adjusting for the other treatments, also testing for a potential interaction. Detailed statistical analyses plans for each endpoint will be developed prior to analysis as a part of the data control work package of DO-HEALTH. The plan will define statistical models for each endpoint and contain printouts of SAS procedures necessary to perform the analysis. All necessary SAS procedures will be pre-programmed and the code will be kept as electronic files to be used in the final analyses.
Planned Subgroup Analyses	To test whether vitamin D_3 and/or omega-3 fatty acids (EPA+DHA) supplements and/or the simple home exercise program reduce the risk of primary and secondary endpoints differentially *by* gender, age (70-84; 85+), body mass index, baseline physical activity, baseline serum 25(OH)D levels, baseline PUFA levels, previous fall (last year), previous fracture (last 10 years), FRAX – estimated absolute fracture risk, baseline symptomatic knee OA, and baseline calcium and protein intake (diet +

	supplements).
Dietary supplements (study intervention)	Each capsule of active study intervention contains either 1000 IU of Vitamin D_3 in the form of crystalline cholecalciferol in a medium chain triglycerides and/or 500 mg of eicosapentaenoic acid (EPA) plus docosahexanoic acid (DHA) in a ratio of 1:2. Placebo capsules contain high oleic sunflower oil replacing vitamin D_3 and/or omega-3 marine fatty acids. **Daily dose of study intervention:** 2 capsules **Route of administration:** orally – preferentially with dinner - or - when participants take all of their interventions (breakfast or lunch or even at bedtime) **Duration of administration:** 3 years All study intervention is provided by DSM and packaged in plastic bottles containing 62 capsules each (one-month supply). Each participant is supplied with 12 bottles once a year at the end of the clinical visit. **Study capsules are to be stored at room temperature between 15 and 25 Celsius degrees.** To avoid unpleasant after taste and thereby unblinding, capsules will be coated so that the capsule will dissolve only in the jejunum.
Justification of the dose of vitamin D and omega-3 fatty acids supplements and the exercise program	**Dietary supplement of 2000 IU Vitamin D per day** – In DO-HEALTH pilot trial (7), 2000 IU vitamin D compared to 800 IU vitamin D per day reduced hospital readmission by 39%, fall-related injuries by 60%, and severe infections by 90%. Further, 2000 IU vitamin D raised 25-hydroxyvitamin 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 assessments(7). Moreover, in another pilot study to DO-HEALTH where we pooled the source data of 11 double-blind randomized trials (8), fracture reduction was only significant at the highest intake quartile of vitamin D (792-2000 IU/day).The safety of 2000 IU vitamin D per day is further supported by our benefit-risk analysis where a safe upper intake level of 10,000 IU of vitamin D per day was estimated (9). In addition, the newest recommendation by the Institute of Medicine increased the safe upper limit for vitamin D intake from 2000 to 4000 IU per day (10). **Dietary supplement of 1 g omega-3 fatty acids per day** – Is recommended by the American Heart Association for cardio-protection and was beneficial with respect to secondary cardiovascular prevention in one trial (11). Because the optimal ratio of EPA to DHA is unknown (12, 13) we have selected a 1:2 ratio of EPA (eicosapentaenoic acid) to DHA (docosahexaenoic acid). Health authorities recommend 400 mg to 1 g/d for cardio protection. A total dose of 850 mg/d was used in the GISSI-Prevenzione Trial (EPA to DHA ratio, 1:2) (11) and AREDS (EPA to DHA ratio, 2:1)(14), and a dose of 1.8 g/d of EPA was used in JELIS (15). Given that the average intake of EPA+DHA is 100 to 350 mg/d in many parts of Europe (16, 17), the proposed intervention of 1 g/d is expected to increase the average participant's omega-3 intake by a factor of 3 to 10. Health risks associated with marine omega-3 fatty acids are believed to

	be minimal and doses of up to 3 g/d are generally recognized as safe. Although omega-3 fatty acids have potential antithrombotic effects, systematic reviews of data from small, short-term trials suggest that omega-3 fatty acid supplements do not increase the risk of clinically significant bleeding at doses of up to 4 g/d, even in combination with anticoagulant interventions such as aspirin or warfarin (18, 19). **For the exercise intervention**, we will test a simple exercise program that was validated in the DO-HEALTH pilot trial (7). The simple well-defined exercise home program was successful in reducing the rate of falling by 25% (significant) and the rate of fracture by 56% (95% CI: −82% to 9%; *P* =0.08; pilot study was not powered for fracture reduction) in the first year after hip fracture. Seniors 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)(7). The program will be instructed by a physiotherapist at the baseline visit. **DO-HEALTH will develop a motivational and animated video to instruct the Strength exercise program as the main exercise intervention in DO-HEALTH**. In addition, a paper format of the program will be developed. For the **exercise control group**, a similar animated video will be designed for a **Flexibility exercise program**, which will serve as a high quality control intervention. The **Flexibility exercise program** will focus on joint mobility rather than strength and balance. **Participants are asked to do the exercise programs 3 times per week for 30 minutes**. The study staff and participants will be informed that both interventions are beneficial and all in-person contacts (every 3 month) will be used as a standardized motivational opportunity. The physiotherapist who will instruct the exercise program during the baseline visit is not a part of the assessment team in DO-HEALTH. Seniors who want to step up the intensity of the program will be suggested to repeat the whole program, thus increasing the training time. *We have not included calcium supplementation as a component of the intervention* as at the higher dose of vitamin D intake, additional high dose calcium supplementation (1000+ mg) was not beneficial for fracture reduction in a pilot study of DO-HEALTH pooling the source data of 11 double-blind RCTs (8); and calcium supplements given without vitamin D may increase the risk of hip fracture (20), the risk of myocardial infarction (21) and the risk of kidney stones (22). In the participant information, we encourage to cover calcium needs sufficiently from dietary sources (1000 mg per day). A personal intake of calcium supplements will be allowed in DO-HEALTH up to a dose of 500 mg per day.
Feasibility and Strengths	**Feasibility of DO-HEALTH clinical visit burden and recruitment potential** has been established in a pilot trial among the target population (Zurich Disability Prevention Trial: NCT01017354); and **optimal dosing and safety of the interventions** have been established in 2 pilot trials (7, 23), one pooled analysis of 11 double-blind RCTs (8), a benefit risk assessment (9) and 3 meta-analyses (3, 8, 24). DO-HEALTH has **more than 80% power for the primary endpoints and all secondary endpoints**. Further, DO-HEALTH will measure 25(OH)D and PUFA levels and extended biomarker

	studies in all participants at all 4 clinical visits. Also, a cost-benefit analysis will be included in DO-HEALTH. **All 3 interventions have a high-quality control group** (placebo for the two nutrients and Flexibility exercise program intervention for the exercise control group).
Limitations	The trial will test only one dose of each agent rather than examining multiple doses. However, the dose for each agent was chosen on the basis of an extensive review of available evidence, including several pilot studies (7, 23) and meta-analyses (3, 8, 24) (see background section page 29). Because the trial population is older, the results may not be transferable to younger men and women
Endpoint ascertainment and validation / Monitoring	To validate self-reported study endpoints, the recruitment centres will send a medical release form to obtain confirmation from general practitioners and/or hospital/physician records for all endpoints reported during the last three months of participants' follow-up time (participants authorize DO-HEALTH staff to do so with their written informed consent). The request to physicians will be accompanied by a copy of the consent form and a cover letter explaining that the participant is enrolled in the DO-HEALTH clinical trial expressing the scientific importance of record validation to the treating physician/ hospital. If there is no response within 1 month, a second request will be mailed, followed by a phone call by the respective DO-HEALTH recruitment centre (all efforts will be documented in the central data base). After records are obtained, the central data management is notified and a de-personalized copy of the records only indicating the subject ID is faxed to the DO-HEALTH coordinating centre at the University of Zurich. There, an Endpoints Committee of Physicians, blinded to the randomized treatment assignment, will review the file and, and using a pre-defined protocol (diagnostic cards), will confirm or disconfirm the case. DO-HEALTH ensures that GCP guideline compliance is a key quality target. Each recruitment centre will be monitored repeatedly and reports will be sent to the Data Safety and Monitoring Board.
Good Clinical Practice (GCP) statement	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, and ICH-GCP as well as all national legal and regulatory requirements.
Diet assessment for subgroup analyses (calcium/protein intake)	We developed an electronic food frequency questionnaire (FFQ) as a tool that is consistent across countries in DO-HEALTH and targeted at the senior population. The development was led by Prof. Walter Willett (collaborator and advisor to DO-HEALTH) and Simonetta Salvini (diet expert and trainee of Prof. W. Willett at the Dept. of Nutrition at Harvard School of Public Health) in collaboration with the DO-HEALTH coordinating centre at the University of Zurich (Prof. Heike Bischoff-Ferrari).

5. Background

5.1. General Background on Aging

The European population is ageing, and the number of seniors aged 70 and older is predicted to increase by 40% by 2030 (25-29), as will the number of seniors with age-related chronic diseases (24, 25). Thus therapeutic interventions that are effective, affordable, and well-tolerated in the prevention of chronic disease at older age are urgently needed and will have an outstanding impact on public health as a whole. Among the most promising interventions that meet these requirements are vitamin D, marine omega-3 fatty acids, and physical exercise. However, their individual and combined effects have yet to be confirmed in a large clinical trial. The urgent need for a large clinical trial to establish evidence for important health endpoints has been confirmed at the 2010 European Parliament Hearing on Vitamin D (30).

Need for clinical trials in the senior population

Exclusions based on older age are frequently used in clinical trials (31), especially of interventions tested in the treatment of cardiovascular disease (32), osteoarthritis (33), and cancer (34, 35). As seniors experience the most morbidity and mortality from acute and chronic diseases their exclusion from clinical trials is problematic since findings from younger adults cannot be extrapolated to the seniors. Seniors older than 75 and those who classify as pre-frail, i.e. ones who experienced a fall or fracture, are the least likely to be included in clinical trials (31). In part, this is driven by the concern that co-morbid conditions or competing risks of disease may camouflage a benefit of the intervention or that the risks of an intervention may be increased or less controllable in a senior individual with an elevated likelihood of impaired kidney and gastro-intestinal function. Further, impaired cognitive function is a concern in an elderly trial population as adherence to the study intervention may be decreased and adverse events such as falls tend to be forgotten (36). Importantly, since conclusions reached in clinical trials of young people cannot be extrapolated to seniors, inclusion of seniors in clinical trials is justified and urgently needed because the senior segment of the European population is growing (25-29) as is the absolute number of individuals with age-related chronic diseases (37, 38).

Prevalence of vitamin D deficiency, low omega-3 fatty acids intake and physical inactivity in the senior population

The 2010 Hearing on vitamin D at the European Parliament recognized broad deficiency of vitamin D: 50% of seniors had 25-hydroxyvitamin D threshold level below 50 nmol/l and 70% – below 75 nmol/l. **The European Parliament reviewed the evidence and acknowledged the urgent need for more evidence and better consumer choices for supplementation** (30)**.** Notably, in the European SENECA study 36% of senior men and 47% senior women had 25(OH)D serum concentrations below 30 nmol/l (consistent findings come from more recent studies (39-41)), which is a cause of concern as *optimal chronic disease prevention was suggested to occur at much higher levels of at least 75 nmol/l* (1) (Figure 2). Seniors are particularly vulnerable, because their skin is less able to synthesize vitamin D in response to solar radiation and because they tend to avoid the sun (42-44). Also, dietary sources of vitamin D are rare and largely limited to fatty fish. 800 IU vitamin D per day, the dose that is recommended today for the prevention of falls (45-48), and fractures (49) translate into more than one fish meal daily (fatty fish such as salmon), let alone a higher dose

of 2000 IU vitamin D per day, which has been suggested as desirable dose for multiple health endpoints (1). Similarly, low omega-3 fat intake and lack of exercise are acknowledged public health concerns at the European level and especially in the senior population (37, 38, 50). Notably, despite the recognition of broad deficiency of vitamin D, omega-3 fats, and exercise, broad public recommendations cannot be substantiated to date because definitive data on health benefits and risks of the 3 interventions individually or in combination are lacking.

5.2. Vitamin D

Skeletal benefits of vitamin D

Recent recommendations by the International Osteoporosis Foundation (IOF) (46), the US Endocrine Society (47), and the Institute of Medicine (IOM) (51) agree that vitamin D supplementation is beneficial for bone health, however the 25-hydroxyvitamin D threshold recommended for bone health varies between 50 and

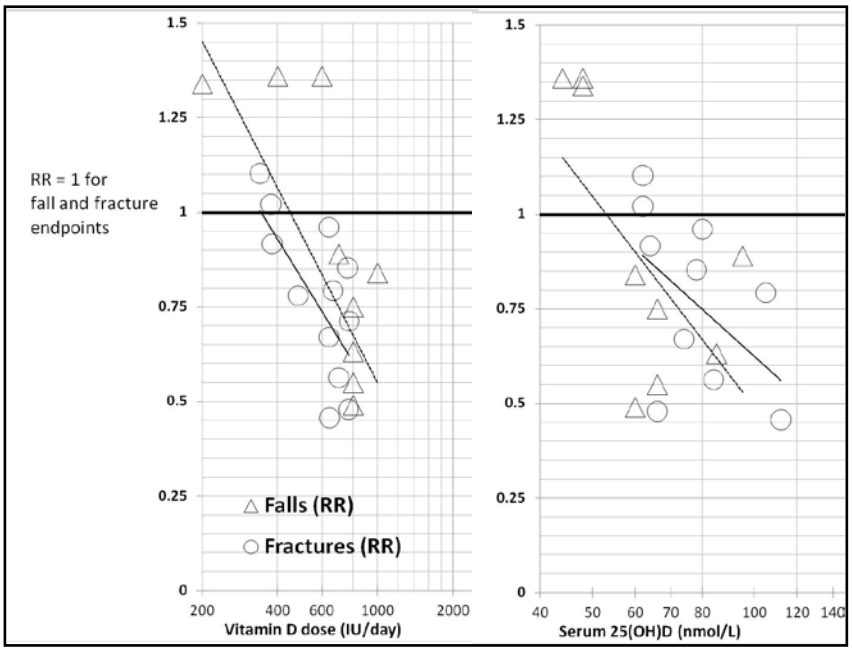


Figure 1. Effects of vitamin D and serum concentrations of 25(OH)D on falls and fractures in senior populations *(Adapted from Bischoff-Ferrari et al.(1)).*

Circles represent relative risks (RRs) from 12 double-blind RCTs on vitamin D supplementation and non-vertebral fracture risk as summarized in a 2009 meta-analysis (3). Triangles represent RRs from 8 double-blind RCTs on vitamin D supplementation and fall risk as summarized in a 2009 meta-analysis (5). Trend line is based on series of effect sizes (circles/triangles). Data from these double-blind RCTs suggest that the benefit of vitamin D is increased with a higher dose and a higher achieved 25-hydroxyvitamin D level in the treatment groups.

75 nmol/l (20-30 ng/ml) (46, 51). Further, there is disagreement in recommendations regarding the benefit of vitamin D on fall prevention. While the IOM concluded that the evidence of vitamin D on fall prevention is inconsistent, the 2011 assessment of the Agency for Healthcare Research and Quality (AHRQ) for the U.S. Preventive Services Task Force (48), the 2010 American Geriatric Society/British Geriatric Society Clinical Practice Guideline (52), the 2010 assessment by the IOF (46), and the 2011 recommendations on vitamin D by the Endocrine Society (47) identified vitamin D as an effective intervention to prevent falling in older adults. Similarly, recent meta-analyses came to conflicting findings on the benefit of vitamin D on fracture prevention (3, 53-56). One explanation for the difference in vitamin D recommendations may be the dose-dependent benefit of vitamin D suggested in two recent meta-analyses and one pooled analysis of double-blind randomized controlled trials, where the effect of vitamin D on fall (5) and fracture(3, 8) prevention depended on the dose and 25-hydroxyvitamin D level achieved.

Non-skeletal and non-muscular benefits of vitamin D

The November 2010 guideline by the IOM states that there is no definite evidence to recommend vitamin D for its potential non-skeletal benefits as data from large trials are lacking (51). The justification for a large trial is, however, supported by significant advances in our knowledge from mechanistic studies, small clinical trials, and observational studies that strongly suggest benefit of vitamin D on:

(1) Heart health and blood pressure reduction: three small clinical trials showed that vitamin D/UVB-irradiation reduced blood pressure in postmenopausal women by 6 to 9 mmHg (57-60), including one pilot trial performed in preparation of DO-HEALTH (23). A cardio-vascular effect of vitamin D is further supported by mechanistic studies documenting that many cell types, including vascular smooth muscle cells, endothelial cells, and cardiomyocytes produce 1α-hydroxylase or express VDR (60-63). $1,25(OH)_2$ D, acting through VDR, exerts a multitude of vasculoprotective effects, including inhibition of vascular smooth muscle cell proliferation by modulating calcium influx into cells (64) and increasing matrix Gla protein, an inhibitor of vascular calcification (65). Vitamin D also regulates the renin-angiotensin-aldosterone system (RAAS) via suppression of renin gene expression and biosynthesis (66, 67). Through its effect on the RAAS, vitamin D is linked to regulation of volume homeostasis and blood pressure (68). Similarly, vitamin D deficiency may be

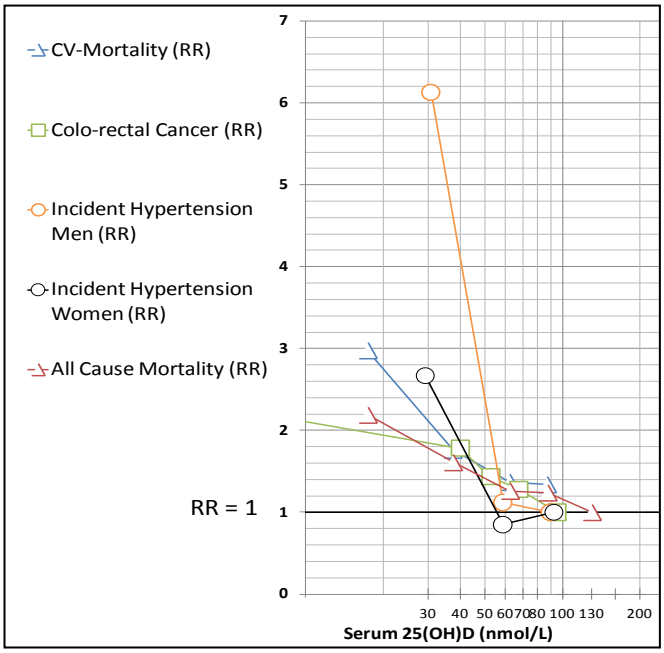


Figure 2. Serum 25-hydroxyvitamin D concentrations of 75 nmol/l confer the lowest risk of cardiovascular disease, mortality and colorectal cancer *(Adopted from Bischoff-Ferrari et al.(1))*.

Lines relate to data from epidemiologic studies on the RR of incident hypertension (2), all-cause (4) and cardiovascular (4) mortality and colorectal cancer (6) based on categories of median 25-hydroxyvitamin D levels. For colorectal cancer (green line), we included a quantitative meta-analysis of 5 studies (6). Based on this summary of non-skeletal endpoints of public health significance, there was a dose-response of better health status with higher median 25(OH)D levels. By visual inspection, the desirable median serum 25(OH)D level to be achieved for all endpoints was approximately 75 to 100 nmol/l.

associated with heart failure through its effect on the RAAS and cardiac morphology. Animal studies have also demonstrated that a lack of vitamin D action leads to hypertension (67) and increased thrombogenicity (69). Clinically, low levels of circulating 25-hydroxyvitamin D (25(OH)D) have been found in subjects with vascular calcification (70), MI (71), stroke (72), heart failure (73), and peripheral arterial disease (74). Low 25(OH)D is also associated with greater carotid intima-media thickness (75, 76) and manifest cardiovascular disease (CVD)(61, 77) in subjects with type 2 diabetes. Moreover, prospective cohort studies of healthy populations show that vitamin D insufficiency, predicts increased CVD risk (78-82) (Figure 2). Vitamin D may also inhibit atherogenesis via anti-inflammatory pathways.

(2) Brain health and cognitive decline: Among 80 seniors referred to a consultative clinic for the assessment of memory loss, 25-hydroxyvitamin D levels were significantly correlated with the mini-mental state examination (MMSE; correlation coefficient r = 0.47; p = 0.006) (83). In the InCHIANTI population-based study conducted in Italy including 858 seniors age 65 years or older the risk of substantial cognitive decline as assessed with the MMSE was 60% higher among participants with severe vitamin D deficiency (serum 25-hydroxyvitamin D levels <25 nmol/l) in comparison with those with sufficient levels of 25-hydroxyvitamin D (≥75 nmol/l) (84) – findings comparable to a recent brain magnetic resonance imaging (MRI) study (85) and another observational study (86). Consistent with association between vitamin D and cognitive health, the VDR is expressed widely in the human brain (87) and vitamin D deficiency during fetal growth leads to major changes in the brain at birth (88). In addition, absence of VDR in knock-out mice has been found to affect emotional (89) and locomotor (90) behavior. Finally, as cognitive decline and disease progression in Alzheimer's disease may be linked to rise in age-related pro-inflammatory cytokines (39, 91, 92), there is reason to study vitamin D (and omega-3 fats and exercise) in prevention of dementia for its anti-inflammatory (93-97), neuro-protective (98-100), and vaso-protective benefit (13, 64-67, 101-106).

(3) Immunity: There is increasing recognition of the role of vitamin D in the immune response to infectious agents, such as tuberculosis bacteria (107) or viral and bacterial infections of the respiratory tract (108, 109). Experimental data suggest that vitamin D inhibits bacterial growth(110) and decreases pro-inflammatory cytokines such as interleukin-6 and tumor necrosis factor-α (TNF-α), decreases C-reactive protein (CRP), and up regulates production of the anti-inflammatory cytokine interleukin-10 (IL-10) (111), *all biomarkers that will be assessed in DO-HEALTH*. Further, the VDR is expressed widely by cells involved in immune response (i.e. T-cells, B-cells, macrophages) (112, 113). In the pilot trial to DO-HEALTH, 2000 IU vitamin D compared to 800 IU vitamin D per day reduced the risk of hospital re-admission significantly by 39% among 173 acute hip fracture individuals. By cause of re-admission, 2000 IU vitamin D per day reduced the rate of severe infection significantly by 90% compared to 800 IU vitamin D per day. Further, a double-blind RCT among Japanese school children found that 1100 IU vitamin D per day compared to placebo reduced seasonal flu rate significantly by 42% plus reduced the rate of asthma attacks in children with asthma (114). Vitamin D has been shown in laboratory studies to inhibit growth of cancer cells by regulating several genes responsible for cell proliferation (115-119) repressing growth factors such as insulin-like growth factor-1 (IGF-1), and epidermal growth factor receptor (EFGR) (120, 121). Recent in vivo and in vitro studies have further suggested that vitamin D signaling is particularly relevant for advanced-stage or high-grade tumors because of its inhibitory effects on angiogenesis, invasion, and metastatic potential (122, 123). $1,25(OH)_2D$ treatment of cancer cells inhibits cell tube formation and tumor growth by repressing vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8) (124, 125). DO-HEALTH may be sufficiently large to be powered for the total cancer endpoint based on a recent double-blind randomized trial, which showed a significant 60% reduction of total cancer risk among 1179 community-dwelling healthy postmenopausal women treated with vitamin D plus calcium compared to placebo over a 4-year follow-up (126).

(4) Glucose-metabolic health: In rodents, administration of $1,25(OH)_2D$ or its analogues enhances vascular reactivity (127), improves insulin sensitivity and insulin secretion (128, 129), and prevents type 1 diabetes (130-132). Results of cross-sectional, case-control, cohort, and small intervention studies in healthy and ill populations suggest favorable effects of vitamin D on impaired glucose tolerance, decreased insulin sensitivity, or type 2 diabetes (62, 133-135); although this was not observed consistently (136). A recent meta-analysis of four case-control studies of ~6500 infants suggests that supplemental vitamin D prevents

type 1 diabetes (OR = 0.71; 95% CI: [0.60 – 0.84]), with higher cumulative doses conferring greater benefit (137); a cohort study found similar results (138). Favorable effects of vitamin D are suggested for kidney function (139, 140) and low 25(OH)D is also associated with greater carotid intima-media thickness (76) and manifest cardio-vascular disease (61, 77) in subjects with type 2 diabetes.

(5) Osteoarthritis: osteoarthritis (OA) is the leading cause of disability in later age (141) and disability due to OA includes pain (142), muscle weakness (143), impaired function, falls (144-146), and fractures (144, 145, 147, 148). Due to the rapidly growing older segment of the population, it has been projected that the percentage of persons with symptomatic OA age 65 or older will almost double in the coming 25 years in the US (149) and Europe (150). Already now, approximately 50% of individuals age 70 years and older have radiographic changes consistent with OA (151), and 30% have symptomatic OA marked by pain in the affected joint (150, 152). Furthermore, a recent prospective study among men and women age 75 or older found that knee pain due to OA was associated with a 26% increased risk of falls and a two-fold increased risk of hip fracture (147). Notably, despite its frequency, OA is a condition that is poorly understood, with no specific treatments and no established means to prevent or reverse the condition (153, 154). One promising secondary prevention strategy in older individuals with symptomatic knee OA may be oral vitamin D supplementation (also omega-3 and exercise – see below). Data from epidemiological studies indicate that increased vitamin D intake and higher 25-hydroxyvitamin D levels (155, 156) may be strategies to prevent progression of knee and hip OA. Vitamin D deficiency is common among individuals with knee OA and lower vitamin D levels are associated with decreased bone density (157), more pain and disability in these individuals (158). Because knee pain in OA may be due to synovitis rather than cartilage loss (159, 160), there is a rationale to consider both vitamin D (93, 94, 97) and omega-3 (161) for their anti-inflammatory and pain reducing benefits. One recent RCT of vitamin D in knee OA and its recently released results suggest a favorable effect of vitamin D supplementation on knee pain(162) with a 20% treatment effect that the trial was insufficiently powered to detect. DO-HEALTH will have a knee OA component roughly 3 times larger than this study and will be sufficiently powered to detect this small effect.

(6) Oral and Gastro-intestinal health: Periodontal disease is the leading cause of tooth loss, particularly in older persons (163-166), and tooth loss is an important determinant of nutrient intake and quality of life (167-169). Similarly, impaired gastro-intestinal function is prevalent in the senior population (170), and correlates inversely with physical activity and quality of life (170-172). One large epidemiological study reported less periodontal disease with higher serum 25(OH)D status(173) and a higher dietary intake of omega-3 fats (174). In one RCT, vitamin D (700 IU/day) plus calcium (500 mg/day) supplementation significantly reduced tooth loss in seniors over a 3-year treatment period (OR = 0.4; 95% CI: [0.2 – 0.9]) (175). Vitamin D and omega-3 fats may reduce periodontal disease through their benefit on alveolar bone (176-183) and anti-inflammatory effect (184-186). The latter may explain the linkage between oral health and coronary heart disease. In one large cohort study remaining teeth predicted in a dose-dependent manner all-cause mortality and mortality from cardiovascular disease and from coronary heart disease ($P < 0.0001$ for all) (187). Seniors with >25 teeth compared to those who had <10 teeth missing had a seven-fold increased risk for mortality from coronary heart disease (187). Regarding gastro-intestinal function, one small trial with 1200 IU vitamin D_3 per day reduced the risk of relapse in subjects with Crohn's disease from 29% to 13%, ($P = 0.06$). In a meta-analysis of prospective data from 5 large cohort studies with a total of 535 cases, persons with serum 25-hydroxyvitamin D levels ≥83 nmol/l had about half the risk of colorectal cancer than those with ≤30 nmol/l (188).

(7) General health/ mortality: The purported multi-organ benefits of vitamin D may explain its benefit on longevity. 25(OH)D levels have been associated with mortality in several epidemiologic studies (78, 189-192), most of which suggested a continuous inverse relationship between increasing values of 25(OH)D and a lower risk of mortality. This inverse association between higher 25(OH)D level and lower risk of mortality is supported by a 2007 meta-analysis of 9 randomized controlled trials documenting a 7% significant reduction of mortality with vitamin D supplementation compared to control (placebo or calcium) (193). A similar risk reduction was observed in one large RCT, the Women's Health Initiative trial (hazard ratio for total mortality was 0.91; 95% CI: [0.83 – 1.01]) (194). Notably, however, in two observational studies, a U-shaped relationship has been described with an increased risk of mortality both at low (< 50 nmol/l) and very high levels of 25(OH)D (> 220 nmol/l) (191, 195). The threshold that conferred the maximum risk reduction for mortality across all studies was 75 to 110 nmol/l (9) (Figure 2).

5.3. Omega-3 Fatty Acids

(1) Bone and muscle health: Omega-3 fatty acids may have an impact on bone (196) and muscle (197) but long-term human data are limited (198).

(2) Heart health and blood pressure reduction: Marine omega-3 fatty acids have shown considerable promise for the secondary prevention of cardiovascular disease in high-risk settings (199-201); and several mechanistic pathways support cardio-protective properties of the nutrient, including reduced susceptibility to atrial and ventricular arrhythmias, anti-thrombotic, hypotensive and anti-inflammatory effects, retarded growth of atherosclerotic plaques, slowing of heart rate, and promotion of nitric oxide induced endothelial relaxation (13, 101, 102, 104-106, 202). The largest trial, the GISSI-Prevenzione trial, with 850 mg fish oil and/or 300 mg vitamin E supplements per day demonstrated significant reductions in total mortality (20%), cardiovascular death (30%), coronary/cardiac mortality (35%), and sudden death (45%) with fish oil, while there was no benefit for Vitamin E (199).

(3) Brain health and cognitive decline: Several observational studies report inverse associations of high fish intake and cognitive decline or risk of dementia,(203-209) although these findings are not uniform across studies (210-213). One larger trial (the OPAL Study) in 867 cognitively healthy adults, aged 70-79 years, showed no benefit of 200 mg EPA plus 500 mg DHA on cognitive decline compared to olive oil over a 24-month follow-up (214). The authors suggest from their findings that further trials are needed in a population at higher risk of cognitive decline, also including seniors at older age having low fish intake.

(4) Immunity: Omega-3 fatty acids have been shown to suppress biosynthesis of compounds that promote inflammation and immune cell function (215). Clinically, higher omega-3 fat intake was associated with reduced inflammation in critical illness (216), rheumatoid arthritis (217) and asthma (218). Many in vitro and animal studies indicate that omega-3 fatty acids (EPA and DHA) can inhibit carcinogenesis by suppressing compounds that promote inflammation, cell proliferation, and angiogenesis and interfere with apoptosis and immune cell function (215). Animals fed a diet rich in omega-3 fats experienced decreased incidence and proliferation of prostate, breast, colon, and pancreatic tumors (215, 219, 220). Relationships between marine omega-3 fatty acids and cancer incidence has also been examined in observational studies of initially healthy populations; risk reduction was found for colorectal, prostate, and breast cancer in several studies, but null results and even increased risks were found in others (221, 222). Using vitamin D and omega-3 fatty acids together may help maximizing cancer prevention because they act through different pathways of

carcinogenesis. For example, vitamin D down regulates growth hormones and suppresses their proliferative activity in many cancers (121, 223, 224), while omega-3 fatty acids may enhance production of free oxygen radicals and lipid peroxides leading to apoptosis in malignant cells (225-227).

(5) Glucose-metabolic health: Benefits of omega-3 fatty acids on insulin sensitivity (228) has been suggested in animal models (229) and small clinical trials (230, 231), although mixed findings from small trials have been reported in a recent meta-analysis (232).

(6) Osteoarthritis: Because knee pain in OA may be due to synovitis rather than cartilage loss (159, 160), there is a rationale to consider both omega-3 fatty acids (161) and vitamin D (23, 93, 94, 97) for their anti-inflammatory and pain reducing benefits. A recent study (Baker and Felson et al poster, ACR 2009) reported a strong association of knee synovitis diagnosed by contrast enhanced MRI with fasting omega-3 fatty acid plasma levels.

5.4. Home-Based Physical Exercise Program

(1) Bone and muscle health: Physical exercise has been shown to improve bone density (233), functional performance, and reduce falls among seniors, even as an unsupervised program (115, 234-237), but fracture reduction data are lacking from randomized controlled trials. In the DO-HEALTH pilot trial, a well-defined exercise home-based program (the same program will be tested in the DO-HEALTH trial), was successful in reducing rate of falling by 25% (significant) and rate of fractures by 56% in senior hip fracture individuals (95% CI: 82% to 9%; P = 0.08; pilot study was not powered for fracture reduction) (234). Seniors 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)**.**

(2) Heart health and blood pressure reduction: Exercise is an important strategy to reduce blood pressure. A meta-analysis of controlled trials of isometric exercise on resting blood pressure included 5 trials and 122 subjects. Isometric exercise for <1 h per week reduced systolic blood pressure by 10.4 mm Hg and diastolic blood pressure by 6.7 mm Hg (238). Exercise has been associated with reduced cardiovascular risk (239) and risk of type 2 diabetes (240). Using exercise, vitamin D and omega-3 fatty acids together may help maximize cardiovascular disease prevention because they act on different pathways involved in atherothrombosis; omega-3 fats may have a stronger favorable effect on lipids, while exercise and vitamin D may exert a greater influence on blood pressure through distinct mechanisms.

(3) Brain health and cognitive decline: A 2010 meta-analysis of 15 available prospective cohort studies found that seniors who performed a high-level and low-to-moderate level exercise were significantly protected against cognitive decline with a risk reduction between 35 and 38% (241). Using exercise, vitamin D, and omega-3 fatty acids together may help maximize prevention of cognitive decline because they are likely to protect the brain through different pathways: omega-3 fats and exercise may have stronger favorable neuro-vascular effect, while vitamin D may exert greater benefit on cognitive health and executive function (242); exercise may be most beneficial for memory function.

(4) Immunity: There is evidence that moderate exercise may stimulate immune function. Moderate exercise resulted in a 20% increase in serum immunoglobulins in a controlled trial of sedentary women (243) and was associated with reduced risk of community-acquired pneumonia in another study (244). Also, higher physical activity and exercise has been associated with lower risk of cancer in men and women (245).

(5) Glucose-metabolic health: Based on observational studies and small clinical trials, exercise has shown considerable potential in the prevention of impaired glucose tolerance and type 2 diabetes (246-248).

(6) Osteoarthritis: Small clinical trials suggest that physical exercise reduces pain and improves activity in subjects with knee osteoarthritis (249). DO-HEALTH will be the first large trial to investigate the benefits of the 3 interventions on symptomatic knee OA. Approximately 30% of individuals aged 70 years and older have symptomatic knee OA (150, 152). Using exercise, vitamin D and omega-3 fatty acids together may help maximize pain-reducing benefits in knee OA because they act on different pathways involved in joint health.

(7) Oral and Gastro-intestinal health: In a recent study of 983 participants of a weight loss program, higher physical activity was associated will less abdominal pain and less gastro-intestinal symptoms (250); other epidemiologic data describe an inverse association between physical activity and the risk of colorectal cancer (251).

6. Study Objectives

6.1. Broad Goals

The DO-HEALTH trial is designed to establish evidence for the role of vitamin D, omega-3 fatty acids, and a simple exercise program, both individually and as a combined intervention, in chronic disease prevention at older age.

6.2. Objectives

Main Objectives:

- To improve healthy ageing in European seniors
- To reduce healthcare costs via the implementation of effective and broadly applicable disease prevention interventions

Specific Objectives:

- To establish whether vitamin D, omega-3 fatty acids, and a simple home exercise program will prevent disease at older age
- To assess comparative effectiveness and cost-benefit of the interventions

6.3. Scientific Objectives

We conduct a multi-centre, randomized, double-blind, placebo-controlled, 2×2×2 factorial clinical trial (n = 2152; 269/ treatment group) of vitamin D_3 (2000 IU/day), and/ or omega-3 fatty acids (1 g/ day EPA + DHA, ratio 2:1), and/or a simple home-based exercise program (30 min. × 3 times per week) vs. control over 36 months in seniors aged 70 and older. The aim is to assess the role of these agents in prevention of chronic disease in seniors and the contribution of the interventions to healthy ageing.

- To test whether 2000 IU vitamin D reduces risk of chronic disease in seniors compared to placebo.
- To test whether 1 g of marine omega-3 fatty acids (EPA+DHA) reduces the risk of chronic disease in seniors compared to placebo.
- To test whether a simple home muscle strength exercise program reduces the risk of chronic disease in seniors compared to control (the Flexibility exercise program) performed 30 minutes 3 times a week).
- To test whether there is an additive value of the 3 interventions combined as a multi-modal intervention in the reduction of chronic disease in seniors.
- To assess the comparative effectiveness of the interventions and to test whether and to what degree adherence modulates the effect of the 3 interventions on risk reduction of chronic disease in seniors.
- To test whether subgroups of the senior population (by gender, age [70-84; 85+], body mass index, baseline physical activity, baseline serum 25(OH)D levels, baseline PUFA levels, previous fall (in the year preceding enrollment in the DO-HEALTH trial), previous fracture (last 10 years), FRAX –

estimated absolute fracture risk, baseline clinical knee OA, and baseline calcium and protein intake) have a differential benefit from the 3 interventions regarding risk reduction of chronic disease.

- To assess the cost-benefit of the 3 interventions individually and in combination as a multi-modal intervention based on an objective health economic model.
- To improve medical care of seniors by establishing laboratory reference ranges for a large set of common laboratory markers and by extending the WHO FRAX fracture prediction model by including the risk of falling.
- To validate novel biomarkers of bone and muscle functionality and immunity based on their response to the 3 interventions and based on the incidence of musculoskeletal and immunity endpoints.

6.4. Technical and Technological Objectives

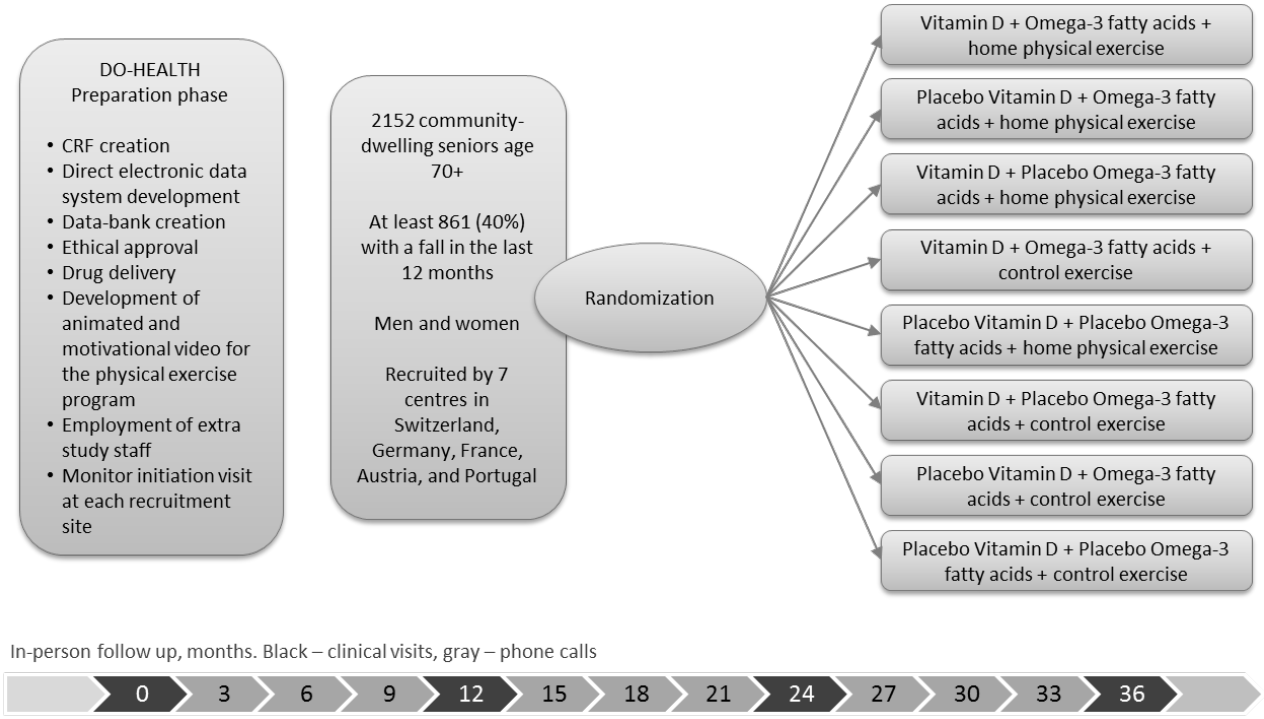
Within the DO-HEALTH clinical trial, the following technical and technological objectives are defined:

- To advance the state of the art in clinical trial data collection and management by creating a complete data system that collects, verifies, safeguards, and provides clinical trial information more easily and efficiently than existing systems.
- To design an animated and motivational exercise instruction video tool for the DO-HEALTH exercise intervention, which can be translated into clinical practice and as an evidence-based home exercise strategy after DO-HEALTH project has been completed. A paper-based version of the instructions will also be created.
- To design a practical web-based and interactive software tool for seniors and health care professionals that will explain how organ-specific DO-HEALTH findings (i.e. heart and bone health) can be translated into personal use and illustrate potential benefits of one, two, or all 3 interventions tested in DO-HEALTH.
- To develop a DO-HEALTH health economic computer model simulating relationships between the 3 interventions and the health outcomes. The model will be designed to allow the interventions to be evaluated for their cost-effectiveness.

7. Study Design

This is a randomized, double-blind, placebo-controlled, 2×2×2 factorial design clinical trial. The trial will be performed at 7 recruitment centers located in 5 countries (Figure 3): Switzerland (University of Zurich, Basel University Hospital, Geneva University Hospital), France (University of Toulouse Hospital Centre), Germany (Charité Berlin), Portugal (University of Coimbra), and Austria (Innsbruck Medical University).

Figure 3. DO-HEALTH study schema



7.1. Primary Endpoints

The DO-HEALTH trial will address five primary endpoints:

Bone: Incidence of non-vertebral fractures over 36-month period. All fracture events will be confirmed by X-ray reports or medical records that describe an X-ray report of the fracture or mention the repair of the fracture. The endpoint will be assessed every 3 month (4 visits, 9 phone calls) in all 2152 seniors. Fracture classification cards will be standardized with the US VITAL trial (detailed fracture classification cards will be described in the fracture assessment manual to DO-HEALTH).

Muscle: Functional decline will be assessed with the Short Physical Performance Battery (SPPB) (252), 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 (252). The SPPB is a brief performance-based test that includes walking speed, repeated chair stands, and a balance test. Its three components are each scored 0 to 4, with 4 indicating the highest level of performance, and are summed up to yield an overall score. The SPPB will be assessed at all clinical visits: at baseline, 12, 24, and 36 months in all 2152 participants.

Cardiovascular: **Systolic and diastolic blood pressure changes** will be assessed at baseline, 12, 24, and 36 months. Blood pressure will be measured after 5-minute rest in a seated position following a standardized protocol validated in a DO-HEALTH pilot trials(23).

Brain: **Cognitive decline** will be assessed using the Montreal Cognitive Assessment (MoCA) at each of the clinical visits: at baseline, 12, 24, and 36 months. The structure of MoCA is similar to that of the Mini Mental State Examination (MMSE) and was found to be more sensitive than MMSE with respect to mild cognitive impairment (253-257).

Immunity: **Rate of any infection.** Incidence of infections will be assessed every 3 months (4 clinical visits and 9 phone follow-ups). Upon each contact, the participants will be asked whether any infection with or without fever has occurred and whether and when a vaccination was performed (i.e. flu vaccination). In case a participant has experienced an infection, a detailed infection questionnaire developed in two pilot trials to DO-HEALTH (258, 259) will be applied. The questionnaire collects information about symptoms, treatment, MD contact and medical assessments. Every case of infection classified as serious adverse event and any case of infection in the last three months of a participant's follow-up time will be confirmed by medical records.

7.2. Secondary Endpoints

Bone:

- **Incidence of hip fractures over 36-month period.** Diagnosis of a hip fracture will be confirmed by X-ray reports or medical records mentioning an X-ray report or fracture repair. The endpoint will be assessed every 3 month (4 visits, 9 phone calls) in all 2152 seniors. Hip fractures include femoral neck and trochanteric fractures (detailed fracture classification cards will be described in the fracture assessment manual to DO-HEALTH).
- **Incidence of vertebral fractures (vertebral morphometry) over 36-month period.** Diagnosis of a vertebral fracture will be performed based on iDXA vertebral morphometry assessment among 1502 participants at four recruiting centers equipped with iDXA machines (Zurich, Berlin, Toulouse, Coimbra). All vertebral bodies of the thoracic and the lumbar spine will be 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 will be classified according to its etiology (for example osteoporotic, traumatic, degenerative, etc.). The assessment of prevalent and incident vertebral deformities based on iDXA morphometry will be blinded to treatment and performed at one specialized centre (Charité, Felsenberg). Results will be sent and included in the central data base at the coordinating centre. The endpoint will be assessed at all clinical visits (baseline, 12, 24, and 36 months) among 1502 participants. We will assess the incidence of mild to severe vertebral deformities, and the incidence of moderate to severe vertebral deformities due to osteoporosis.
- **Incidence of total fractures**: any new non-vertebral – see primary endpoint - PLUS any new vertebral fracture (mild to severe deformity) based on vertebral morphometry in the subset of 1502 seniors with iDXA measurements over 36-month period.

- **Bone mineral density at the spine and hip based on iDXA measurements** will be assessed in 1502 participants at four recruiting centers equipped with iDXA machines. The measurements will be performed during all clinical visits at baseline, 12, 24, and 36 months. Both (right and left) proximal femurs will be scanned at baseline. At follow-ups, only the hip on the side that had the lower bone mineral density value of the total femur region at baseline will be measured. If the hip side with the lower baseline bone mineral density becomes not suitable for DXA measurement (e.g. fracture or endoprosthesis during the course of the study), the other side will be scanned.

Muscle:

- **Rate of falling (rate of any low trauma-, injurious falls, number of participants who fell).** Falling is defined as unintentional 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 will be recorded every 3 months and ascertained with a questionnaire to assess the circumstance of each fall and associated injuries. **The rate of any low trauma falls will be the main fall outcome** supported by additional analyses of injurious falls and the number of fallers (person who sustains a low trauma fall at least once during the course of the trial). Low trauma falls will be defined as falls occurring from a standing height and without the involvement of others or a vehicle. High trauma falls will be defined as falls occurring from a height (i.e. ladder) or stairs (more than two steps), or caused by others (being pushed by a person or an animal) or a vehicle (i.e. being hit by a car or a bike) and will not be included. Injurious falls will be defined as falls that lead to any injury (i.e. skin wound, significant bruising, fracture). Participants will receive a DO-HEALTH diary to note any new health event including a fall. The DO-HEALTH diary serves as a support structure to the 3-monthly reporting of adverse events, and will be collected at each clinical visit. The participant diary will not constitute a direct source of information for endpoint assessment, but might be used *pro re nata* for confirmation of participant reported events.
- **Reaction time and grip strength.** Reaction time will be assessed with repeated chair stands (5 repeats as part of the SPPB(252)), and muscle strength will be assessed with validated grip strength protocol using Martin vigorimeter (260) in all 2152 participants at baseline, 12, 24, and 36 months.
- **Muscle mass in the upper and lower extremities.** Muscle mass will be measured by iDXA in 1502 participants at four recruiting centers equipped with iDXA machines. The measurements will be performed during clinical visits at baseline, 12, 24, and 36 months.
- **Musculoskeletal pain** will be assessed in all 2152 participants at baseline, 12, 24, and 36 months using McGill pain map (261)
- **Dual tasking gait speed** will be assessed in all 2156 participants at baseline, 12, 24, 36 months. This will be assessed by comparing normal gait speed against gait speed if combined with a simple cognitive task over a distance of 10 meters. Gait speed will be 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 (BL: 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) (262). The difference between the two times will be evaluated for this endpoint.

At the study centre UHB (Basel, Switzerland) dual tasking gait speed will be measured using the GAITRite electronic walkway system in addition.

Cardiovascular:

Risk of incident hypertension over 36 months: Blood pressure will be measured after 5-minute rest in a seated position following a standardized protocol validated in one of the DO-HEALTH pilot trials (23). Measurements will be taken at each clinical visit and all cases of incident hypertension that occurred over the 36-month follow-up period will be recorded. Incident hypertension is present if the SBP is ≥140 mm Hg or the DBP is ≥90 mm Hg. In addition, as we see participants only at yearly intervals in clinical visits, a new chart-based diagnosis of incident hypertension and/ or a report of new use of anti-hypertensive drugs will count as incident hypertension. Incident hypertension will be confirmed either (1) based on blood pressure measurement taken during the clinical visits, (2) or a new diagnosis by a treating physician or report of a new use of anti-hypertensive drug.

Brain:

- **Mental health decline and incidence of depression** will be assessed at baseline, 12, 24, and 36 months using an *excerpt* from the Geriatric Depression Scale (GDS)
- **Dual tasking gait variability** will be 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 centre (Basel University Hospital). Seniors will perform 4 walking tasks with and without cognitive challenge (263).

Immunity:

Rates of any upper respiratory infection, incident flu-like illness, incident severe infections that lead to hospital admission over the 36-month period. This will be based on an infection protocol applied every 3 months (see primary endpoint). Infections that lead to hospital admission will be confirmed by medical records and type of infection will be confirmed by the Independent Physician Endpoint Committee.

Bone/cartilage:

- All participants will be evaluated for prevalent knee osteoarthritis (OA) at baseline according to the modified American College of Rheumatology (ACR) criteria (264). According to the modified ACR clinical criteria, prevalent and incident symptomatic knee OA will be diagnosed in participants who report knee pain on most days of the preceding week **PLUS** at least 3 of 6 criteria:
  1) Crepitus based on MD exam
  2) Age 50+
  3) Stiffness < 30 minutes
  4) Bony tenderness based on MD exam
  5) Bony enlargement based on MD exam
  6) No palpable warmth based on MD exam

  The ACR criteria for knee OA have been modified for DO-HEALTH based on the advice of Prof. D. Felson, who is a leading expert in osteoarthritis assessment and epidemiology, and who is the PI of

the Framingham osteoarthritis study. The bony enlargement criterion was excluded due to its poor reproducibility.

- **Severity of knee pain** will be assessed in all 2152 seniors with the Knee injury and Osteoarthritis Outcome Score (KOOS (265)) at baseline, 12, 24, and 36 months. The endpoint will be assessed in all seniors and specifically in seniors with symptomatic knee OA. In addition, the **rate of knee buckling**, **total number of joints with pain**, and **NSAID use** will be assessed at baseline, 12, 24, and 36 months in all seniors and seniors with symptomatic knee OA.

Dental:

- **Decline in oral health** will be assessed in all 2152 participants at baseline, 12, 24, and 36 months with the Geriatric Oral Health Assessment Index (GOHAI (266)) questionnaire.
- **Tooth loss** will be assessed in all 2152 participants at baseline, 12, 24, and 36 months by tooth count by the study MD including all teeth (own and all). Tooth counts will be instructed as part of the DO-HEALTH study staff training. Tooth counts will be performed by the study MD will be validated with tooth prints at one recruitment centre (University of Zurich).
    - In addition, we will ask at all 3 months in person contacts whether the participant lost a tooth (clinical visit and phone calls)
        - At baseline, we ask how many own teeth without wisdom teeth are left (maximum of 28)
        - At baseline, we ask how many teeth are missing and how many were replaced (removable or fixed)
    - We will examine the number of teeth (own and all) at baseline, 12, 24, and 36 months with a simple schema noting “T” for tooth present, “M” for tooth missing, “RR” for tooth replaced and removable, “RF” for tooth replaced and fixed

8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8
8	7	6	5	4	3	2	1	1	2	3	4	5	6	7	8

Gastro-intestinal: **Gastro-intestinal symptoms** will be assessed in all 2152 participants at baseline, 12, 24, and 36 months using Rome III diagnostic questionnaire for the adult functional gastro-intestinal (GI) disorders(267).

Glucose-metabolic:

- **Fasting blood concentration of glucose and insulin** will be measured in all 2152 participants at baseline, 12, 24, and 36 months. Insulin sensitivity and beta cell function derived from indices of fasting glucose and insulin concentrations will be calculated using quantitative insulin-sensitivity check index (QUICKI), and the HOMA index (268).
- **Body composition and fat mass** will be measured by iDXA in 1502 participants recruited by four centers equipped with iDXA machines. The measurements will be performed during clinical visits at baseline, 12, 24, and 36 months. We will assess trunk and upper and lower extremity fat mass.

Kidney: Decline in kidney function. Blood creatinine levels and estimated glomerular filtration rate will be measured in all 2152 participants at baseline, 12, 24, and 36 months with the Cockcroft-Gault formula (269).

Global Health:

- **Quality of life** will be evaluated using the EQ5D-3L questionnaire in all 2152 participants at baseline, and every 6 months afterwards (during 6-, 18-, 30-month phone calls, and at 12-, 24-, and 36-month clinical visits).
- **Incident frailty** will be assessed with the Survey of Health, Ageing and Retirement in Europe Frailty Instrument (SHARE-FI) (270) in all 2152 participants at baseline, 12, 24, and 36 months. SHARE-FI closely resembles the traditional Fried's variables (271, 272), 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** will be measured by the PROMIS-HAQ at baseline, 12, 24, and 36 months(273)**.** We will assess the risk of at least one new limitation in 20 activities of daily living (we will assess 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** will be assessed every 3 months. Mortality will be confirmed by medical record or death certificate; cause of death will be established from medical records or death certificate and classified as follows: cardiovascular, cancer, severe infections, or other.

The application of all questionnaire and survey instruments mentioned in the synopsis and in Sections 7.1 and 7.2 will be described in the DO-HEALTH manual and instructed in the staff training sessions.

Biomarker Endpoints

DO-HEALTH trial will include biomarker endpoints to support primary and secondary endpoints of the study.

The following biomarkers will be measured in all 2152 participants at baseline, 12, 24, and 36 months:

Bone: Calcium, phosphate, 25(OH)D, intact PTH, urinary calcium/creatinine ratio (second spot urine), Beta-Crosslaps serum, P1NP, sclerostin

Muscle: myostatin

Cardiovascular: Troponin T, NT-proBNP, homocysteine, CK, cholesterol, HDL-cholesterol, triglycerides

Inflammation: high sensitivity-CRP, IL6

Gastro-intestinal: ALT, AST, gGT, alkaline phosphatase, bilirubin

Glucose-metabolic: fasting glucose, insulin

Kidney: serum creatinine; calcium/creatinine ratio in second spot urine, serum urea, uric acid

Global Health:
Ions: sodium, potassium, chloride, magnesium

Proteins: total protein, albumin, ferritin, transferrin
Hormones: TSH, fT4, fT3, cortisol
Vitamins: folic acid, vitamin B12, 25(OH)D

Adherence to treatment: serum 25(OH)D measured by two methods (automated assay and gold standard HPLCMS/MS). Plasma PUFA concentrations (EPA, AA, DPA, DHA) measured by a sensitive and selective assay based on gas chromatography coupled to mass spectrometry detection (GC-MS).

The following biomarkers will be measured in the participants from two study centers (Zurich and Basel) at baseline, 12, 24, and 36 months:

Inflammation: TNF-α, IL10, IL-17, IL-22

Cellular immunity: CD3, CD4, CD25, CD127

7.3. Exploratory Endpoints

The DO-HEALTH trial will assess several exploratory endpoints that support the primary endpoints, but for which the study has limited statistical power. Regarding endpoint validation see Chapter 15. All exploratory endpoints will be assessed over the 36-month follow-up period:

Bone:

- **Incident repeat fracture** defined as more than one incident fracture occurring in the same individual at two different time points. Fractures that occur at the same time point do not count as second incident fractures. Any non-vertebral repeat fractures will be evaluated in all participants, while vertebral fractures and total fractures will be evaluated among participants with iDXA measurements. As all fracture endpoints, the endpoint will be validated by an independent committee of physicians blinded to treatment assignment. All fractures will be confirmed based on X-ray or medical reports.

- **Ancillary Fracture healing study (funding support by the AO Foundation):** Fracture healing is a novel endpoint and DO-HEALTH will be one of the first RCTs to assess fracture healing at 3 levels in individuals with incident major osteoporotic fractures at the arm (shoulder, humerus, forearm) and leg (hip, femur, ankle). The fracture healing study is an ancillary study with pending funding by the AO foundation and the endpoint is co-led by the DO-HEALTH partner at the University of Basel (N. Suhm) and the Coordinating DO-HEALTH Centre (H.A. Bischoff-Ferrari) supported by all DO-HEALTH recruitment partners. Extra staff time / logistics / programming / analyses will be funded by the ancillary funds through the AO foundation.

  We will assess *fracture healing* in all seniors with an incident major osteoporotic fracture at the arm (shoulder, humerus, forearm) and leg (hip, femur, ankle) at the endpoints levels – note that all participants are asked to use the telephone hotline of their respective recruitment centre to report an incident fracture within 7 days (there will be a reminder at each 3 month in person contact):

  (a) *primary fracture healing endpoint*: clinical fracture healing with 3 additional phone calls at 6, 12, 18 weeks after the fracture assessing the PROMIS-HAQ. Notably, all participants will have a baseline

PROMIS-HAQ (baseline visit) as this is our tool to assess ADL at BL 12, 24, 36 month in all participants in DO-HEALTH.

(b) *secondary fracture healing endpoint*: observed functional fracture healing measured with the Short Physical Performance Test Battery and grip strength at regular 12, 24, 36 month visits. We will adjust the analyses for time since fracture.

(c) *exploratory fracture healing endpoint*: radiological fracture healing will be assessed at an independent radiology institute using standard x-rays performed at the local hospitals for early (6-8 weeks) and late (12 to 14 weeks) fracture consolidation assessment. For the independent assessment, all x-rays will be de-personalized by the recruitment centre and will carry only the subject ID when sent to the coordinating centre at the University of Zurich.

Muscle:

- **Incident sarcopenia.** Since there is no consensus on a universally accepted definition of sarcopenia to date, all previously used and newly proposed composite definitions of sarcopenia based on appendicular muscle mass and the SPPB, its components (274) and/or grip strength, will be considered. The assessment will be performed among 1502 participants with DXA measurements at BL, 12, 24, and 36 months.
- **Decline in physical activity.** Physical activity will be assessed by an *excerpt* from the Nurses' Health Study (NHS) questionnaire. The NHS physical activity questionnaire is well validated against incident chronic diseases.

Cardiovascular:

- **Incident major cardiovascular event will be assessed as a composite endpoint** (any event: myocardial infarction, stroke, revascularization procedures of CABG and PCI, incident congestive heart disease (heart insufficiency), cardiovascular mortality); all events will be confirmed by medical records and the Independent Physician Endpoint Committee using pre-defined diagnostic cards.

- **We will also analyze the following individual cardiovascular endpoints:** myocardial infarction, stroke, incident congestive heart disease and cardiovascular mortality. Major cardiovascular endpoints will be assessed at every 3 months follow-up and confirmed by medical records.

Brain:

Incident dementia: Any new diagnosis of dementia (self-reported or proxy-reported) will be confirmed by changes in the MoCA. In addition, every case classified as serious adverse event and any case in the last three months of a participants follow-up time will be confirmed by medical records.

Immunity:

- **Incident cancer** (any, gastro-intestinal, breast cancer in women, prostate cancer in men) will be recorded every 3 months and confirmed by medical records reviewed by the Independent Physician Endpoint Committee based on pre-defined diagnostic cards.

- **Rate of implant infections after total hip or knee replacement due to fracture or osteoarthritis** will be recorded every 3 months and confirmed by medical records reviewed by the Independent Physician Endpoint Committee based on pre-defined diagnostic cards.

- **Rate of gastro-intestinal infections** will be recorded every 3 months. Every case classified as serious adverse event and any case in the last three months of a participant's follow-up time will be confirmed by medical records.

Bone/Cartilage-Arthritis:

Incident symptomatic knee osteoarthritis, incident symptomatic hip osteoarthritis, incident symptomatic hand osteoarthritis will be assessed at 12, 24, and 36 months of follow-up. Incident hip and knee OA will be assessed based on clinical examination (modified ACR criteria for hip (275) and knee OA (264)) and specific questionnaires (KOOS (265), HOOS (276)). The ACR criteria for knee and hip OA were modified for DO-HEALTH after consultations with Prof. D. Felson, who is a leading expert in osteoarthritis assessment and epidemiology, and the PI of the Framingham osteoarthritis study (277-280). Bony enlargement evaluation has been excluded from the knee examination due to poor reproducibility and hip flexion assessment has been excluded from the hip examination due to poor specificity (277-281). Hand OA will be assessed with the *Quick*DASH for hand OA (282) supported by the assessment of painful joints.

- **Composite endpoint: incident symptomatic knee, hip or hand osteoarthritis**
- **Severity of hip pain in those with prevalent symptomatic hip osteoarthritis** assessed by the Hip injury and Osteoarthritis Outcome Score (HOOS (276)), a validated instrument that evaluates hip pain with activities
- **Severity of hand pain in those with prevalent symptomatic hand osteoarthritis** assessed by the *Quick*DASH (282)

7.4. Adherence and Safety Laboratory and Safety Endpoints

DO-HEALTH will evaluate several endpoints to assess adherence to the treatment protocol and the safety of the interventions.

Adherence endpoints include: 25(OH)D serum concentrations at baseline, 12, 24, and 36 months; PUFAs serum concentrations at baseline, 12, 24, and 36 months

General safety laboratory include: simple blood count with RBC, Hb, WBC, Tc, machine differential (granulocytes, lymphocytes) platelet count, erythrocyte indices (MCV, etc.), and reticulocytes at baseline, 12, 24, and 36 months.

Safety endpoints for vitamin D include: serum calcium and serum creatinine at baseline, 12, 24, and 36 months. We also assess incident nephrolithiasis based on participant report and confirmed by medical records.

Potential side effects of the study agents will be assessed on each in person follow-up (3-monthly interval). **For omega-3 fatty acids**, these side effects include gastrointestinal (GI) upset (presence or absence of symptoms of peptic ulcer, nausea, constipation, diarrhea), bleeding (any GI bleed, GI bleeding requiring transfusion, hematuria, easy bruising, epistaxis), skin eruptions, and physician diagnosis of atrial fibrillation

or other irregular rhythms. A potential "unpleasant aftertaste" is not expected with the capsule coating in DO-HEALTH, but would be covered in the assessment of comparative effectiveness where we ask why and why not participants adhere to the study intervention.

7.5. Assessment of protein and calcium intake

We developed the electronic DO-HEALTH Food Frequency Questionnaire (FFQ) targeted at the senior population. 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.

The development of the DO-HEALTH FFQ was led by Prof. Walter Willett (collaborator and advisor to DO-HEALTH) and Simonetta Salvini (diet expert and trainee of Prof. W. Willett at the Dept. of Nutrition at Harvard School of Public Health) in collaboration with the DO-HEALTH coordinating centre at the University of Zurich (Prof. Heike Bischoff-Ferrari).

In the development of the DO-HEALTH FFQ, we used as a stepping stone the GA^{2}LEN FFQ (a European network of excellence in the area of research in allergy and asthma www.ga2len.net) and the Nurses' Health FFQ. The DO-HEALTH FFQ is targeted at the senior population, is structured the same in all DO-HEALTH countries, and is designed by DO-HEALTH Partner FDS as a user-friendly interactive tool. Images of food items were created by B. Gut, the DO-HEALTH partner.

The FFQ is organized in food groups, each including 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.*

Seasonality: for each fruit or vegetable item, we allow subjects to indicate if the consumption is only in season.

Food consumption data collected by the FFQs will be further transformed into nutrient data, applying country specific food composition data (www.eurofir.net).

The diet assessment, instrument development and validation of dietary assessment will be submitted by the DO-HEALTH PI (H. Bischoff-Ferrari) for funding as an ancillary project to DO-HEALTH.

7.6. Study Participants and Treatment Groups and In-Person Follow-up

A total of 2152 seniors aged 70 years and older will be recruited into the DO-HEALTH clinical trial.

DO-HEALTH will enroll seniors aged 70 years and older for their high risk of chronic disease, high risk of vitamin D and omega-3 deficiency, and high prevalence of physical inactivity. To represent the largest part of the senior population, DO-HEALTH will recruit community-dwelling seniors. However, to represent also the pre-frail population at risk of institutionalization, at least 40% of seniors will be enrolled based on a low trauma fall with or without a fracture in the year before DO-HEALTH enrolment. Falls are a hallmark of frailty and functional decline and 40% of all nursing home admissions are due to a fall (283).

Justification of number of participants: The sample size was chosen to have sufficient power for the most critical primary and secondary endpoints (any non-vertebral fracture and hip fracture) and, based on prior experience, the expectation that 68% will complete the entire 3-year study. However, the subjects who drop out early will provide partial data since our analyses will be based on the intention-to-treat principle. Under these assumptions and **an additive effect of the 3 interventions,** as expected from our pilot study (7), we will have **> 90% power for all 5 primary endpoints** (with Bonferroni adjustment for multiple comparisons). **If an interaction between the vitamin D and omega-3 fats interventions is assumed, the power still exceeds 80%.** See detailed power calculations below. Contributing to the statistical power for the most critical fracture endpoints is the fact that at least40% of seniors will be recruited based on a prior fall with or without a fracture, which confers an increased risk of sustaining a fracture endpoint during the course of the trial (see detailed power considerations on page 79).

The participants will be randomly assigned to eight groups of 269 people each including a placebo group and active treatment groups assigned all possible combinations of the study treatment regimens (Table 1).

Table 1. Treatment groups

	Vitamin D_3	Marine Omega-3 fatty acids	Exercise program
Group 1 (n = 269)	Placebo	Placebo	Flexibility
Group 2 (n = 269)	2000 IU/d	Placebo	Flexibility
Group 3 (n = 269)	Placebo	1:2 EPA + DHA, 1 g/d	Flexibility
Group 4 (n = 269)	Placebo	Placebo	Strength
Group 5 (n = 269)	2000 IU/d	1:2 EPA + DHA, 1 g/d	Flexibility
Group 6 (n = 269)	2000 IU/d	Placebo	strength
Group 7 (n = 269)	Placebo	1:2 EPA + DHA, 1 g/d	Strength
Group 8 (n = 269)	2000 IU/d	1:2 EPA + DHA, 1 g/d	Strength

Participants will undergo clinical visits at baseline, 12, 24 and 36 months after recruitment and telephone interviews every 3 months between the clinical visits (9 telephone interviews total, Figure 4). Details on clinical visits and telephone interviews are presented in Section 9.

Figure 4. In-person follow-up timeline in months (black - clinical visits, gray - telephone interviews).

7.7. Eligibility and Exclusion Criteria

Inclusion criteria:

- Age ≥ 70 years
- Mini Mental State Examination Score of at least 24
- Living in the community
- Sufficiently mobile to reach the study centre
- Able to walk 10 meters with or without a walking aid and getting in and out of a chair without help
- Able to swallow study capsules
- Able and willing to participate, sign informed consent (including consent to analyze all samples until drop-out or withdrawal) and cooperate with study procedures

Exclusion criteria:

- Consumption of more than 1000 IU vitamin D/day in the 3 months prior to enrollment, or unwillingness to reduce to 800 IU/d of vitamin D (current standard of care) for the duration of the trial
    i. 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, may be enrolled after a 3-month wash-out period where the maximum daily intake is limited to 800 IU of vitamin D
    ii. Provision 2: an individual who consumed an average vitamin D dose higher than 2000 IU/day in the 3 months prior to enrollment, may be enrolled after a 6-month wash-out period where the maximum daily intake is limited to 800 IU of vitamin D
- Unwilling to limit calcium supplement dose to a maximum of 500 mg per day for the duration of the trial
- Taking omega-3 fat supplements in the 3 months prior to recruitment and or unwilling to refrain from the use of omega-3 supplements for the duration of the trial
- 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
- Current or recent (previous 4 months) participation in another clinical trial, or plans of such participation in the next 3 years
- Presence of the following diagnosed health conditions **in the last 5 years**:
    i. History of cancer (except non-melanoma skin cancer)
    ii. Myocardial infarction, stroke, transient ischemic attack, angina pectoris, or coronary artery intervention
- Severe renal impairment (creatinine clearance ≤ 15 ml/min) or dialysis
- Hypercalcaemia (> 2.6 mmol/l)
- Hemiplegia or other severe gait impairment
- History of hypo- or primary hyperparathyroidism
- History of granulomatous diseases (i.e. tubercolosis, sarcoidosis)
- Severe liver disease
- Major visual or hearing impairment or other serious illness that would preclude participation
- Living with a partner who is enrolled in DO-HEALTH (i.e. only one person per household can be enrolled)
- Living in assisted living situation or in nursing home
- Temporary exclusion: acute fracture in the last 6 weeks
- Epilepsy and/or use of anti-epileptic drugs
- Individuals who fell more than 3 times in the last month
- Osteodystrophia deformans (Paget's disease)
- For study centers in Germany only: persons who are institutionalized / in prison by court order (§40, Abs. 1, Art. 4, "Gesetz über den Verkehr mit Arzneimitteln").

7.8. Recruitment Goals and Recruitment Centers

Based on our previous experience and the results from the feasibility pilot clinical trial conducted in preparation to DO-HEALTH (Zurich Disability Prevention Trial: NCT01017354) we estimate that the recruitment period will take will take 20 months (month 12 to 32 of the study; see Figure 6 Project Timeline on page 56). Due to differences in regulatory and approval procedures in different countries, some centers may start recruitment later than month 12. The latest foreseen deadline to start recruitment is month 14. The trial will recruit 2152 participants at 7 study centers in 5 countries. Study centers and their recruitment goals are provided in the table on the next page.

Study centers were selected based on academic excellence and expertise in randomized trials conducted in community-dwelling and institutionalized seniors.

Study centers/countries were also selected based on their expected high prevalence of vitamin D deficiency, low fish intake, and low level of physical activity. Based on the European SENECA study the expected number of seniors with severe vitamin D deficiency (serum 25(OH)D concentrations below 30 nmol/l) is between 30 and 60% in the selected countries (41). Within the SENECA study, vitamin D deficiency was most pronounced in the Mediterranean countries, as seniors tend to avoid the sun (41). Similarly, low omega-3 fat intake and lack of exercise are acknowledged public health concerns at the European level (37, 38, 50).

Recruitment Centre	Planned number of participants	Actual number of recruited participants
Zurich University Hospital, Switzerland	552	552
University of Geneva, Switzerland	200	201
Basel University Hospital, Switzerland	250	253
Toulouse University Hospital, France	300	301
Charité Clinic, Germany	350	350
Innsbruck Medical University, Austria	200	201
Coimbra University, Portugal	300	301
Total	**2152**	**2159**

Strategies to meet recruitment goals: DO-HEALTH has set a recruitment goal of 25% at each recruitment centre by month 20 (6 month after recruitment start in the last fully approved recruitment centre). **If a centre does not meet its recruitment goal by month 20, the centre will be closed for further recruitment and the recruitment load will be shifted to other centers that showed a recruitment rate beyond their estimate**. Alternatively, if this is not possible, a new recruitment centre (at the University of Dresden) will be opened.

7.9. Recruitment and Screening Methods

Screening and recruitment procedures employed by the DO-HEALTH are presented in Figure 5 (page 53).

Recruitment methods: DO-HEALTH will use 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 to bring information about the DO-HEALTH to the target population and

health care professionals – for direct and indirect encouragement for participation. See DO-HEALTH newspaper advertisement on page 66. The advertisement will contain a contact phone number for each specific recruitment site that potential participants are asked to call for further information (DO-HEALTH telephone hotline – established at each recruitment site). The newspaper advertisement will be considered a standard form of advertisement for the Trial. If a study center finds it necessary to employ different media, advertisement text for these media will be designed and the study center will then proceed in compliance with the local laws and regulations.

Screening methods: When a prospective participant reads the DO-HEALTH information and places a call to the local DO-HEALTH telephone hotline, an *ad hoc* screening interview will be performed, or the study nurse will set up a later phone appointment. For the latter instance, a staff member contacts the prospective participant on the scheduled date and conducts the screening (see section 9.1, Screening Telephone Interview on page 66). Decision about eligibility for baseline visit is made at the end of the interview and all information recorded during the interview, except for name and mailing address, is archived or destroyed depending on the local regulations. Name and mailing address of those who pass the screening interview as potentially eligible are used to mail the participant information and informed consent form. The potential participant is asked to study the participant information plus the written informed consent form for at least 48 hours, call back for any questions and confirm the baseline visit date.

If after the screening interview a person appears eligible and willing to participate, a baseline visit is arranged. The baseline visit should be scheduled at least 4 days after the screening interview to allow mail delivery and at least 48 hours for the individual to study the participant information and written informed consent form. If 2 months after the screening phone call a baseline visit has not been fixed yet, the recruitment team might call the prospective participant to follow up with him/ her.

Figure 5. DO-HEALTH screening and recruitment process *(this schema refers to men and women interested in the study).*

Baseline visit requirements: The participant is asked to confirm his/ her baseline visit no later than 1 week prior to its scheduled time (or the study staff confirms the visit by a phone call to the participant). The participant is asked to bring all current interventions, vitamins, mineral supplements, walking aids to the baseline visit appointment, and to wear comfortable shoes and clothes.

At baseline visit, eligibility will be ascertained through:

- Participant interview (inclusion/exclusion criteria)
- Written informed consent
- Normal serum calcium level (≤ 2.6 mmol/l)
- Creatinine clearance > 15 ml/min
- Sufficient cognitive performance based on Mini Mental State Examination (MMSE minimal score of 24 to be eligible)
- Able to walk 10 meters with or without a walking aid and able to get in and out of a chair without help

After any additional questions of the participant with respect to the study are answered sufficiently, the participant will be asked to sign the informed consent. Once the informed consent is signed, blood will be drawn for express assessment of serum calcium level (safety inclusion criteria) and the prospective participant will be evaluated with the MMSE and a screening walking (nurse walks with the participant) and chair test (nurse observes whether the participant can get in and out of a chair without help) to ensure that minimal requirements for cognitive and physical function are met. Final eligibility of the prospective participant will be assessed by the study MD.

Eligible individuals that give written consent to the study are randomized to one of the 8 treatment groups at the day of the baseline visit (see Section 11.3 for details).

7.10. Participant Information and Written Informed Consent

The participant information and written informed consent was tested in the target population for readability and understanding. All individuals who agree to participate in the study will be required to sign informed consent. They will be presented with the Informed Consent that will describe the following in the participant's national language (see participant information and informed consent for the study):

- Purpose and plan of the research
- Nature and extent of any procedures
- Possible risks and benefits
- Local ethics committee approval
- Right to withdraw consent at any time without discrimination or disadvantage
- Arrangements for responding to adverse events
- Confidentiality and privacy arrangements
- Arrangements for access to information
- Compensation arrangements for travel to the recruitment centre
- Any foreseen future uses of the research results, data, or materials
- Source of co-funding

- Right to be informed about the results of the research
- Arrangements for taking care of the subjects after their participation has ended
- Name of the Principal Investigator of the clinical trial and of the physician responsible for enrolment and follow-up of the subject
- Right to continue being followed-up by the participant's own physician in addition to the clinical trial physicians

In addition to the above-mentioned documents, the participant will receive detailed information about bio-bank procedure and implications (information and consent to bio-bank inclusion). The information about DO-HEALTH bio-bank includes:

- Purpose of bio-bank
- Rights and procedures to eliminate one's samples from the bio-bank at any time without discrimination and disadvantage
- Detailed explanation about sample anonymization

Participants will be presented a separate written informed consent form about sample collection, storage and use in the bio-bank. Consent to bio-bank inclusion is strongly encouraged but not binding for participation to the study.

Study clinical personnel will answer all questions and provide all necessary explanations to the participant in relation to the clinical trial providing **adequate** and **understandable** information, avoiding exhaustive and excessive amount of information to participants, and making sure that the person makes a well-informed and voluntary decision prior to signing the form.

7.11. Early Withdrawals and Losses to Follow-up

DO-HEALTH personnel will make every reasonable attempt to encourage adherence and prevent early withdrawals or losses to follow-up. If a participant misses a clinical visit or a phone interview, every reasonable attempt will be made to re-establish contact. Phone calls and mail reminders will be tried and, if unsuccessful, next of kin and a general practitioner of this person will be contacted in an attempt to re-establish the follow-up or identify the reason for loss to follow up. **To accommodate the participant, the follow-up times will be scheduled with some flexibility (maximum of 14 days before or after the set date) and the clinical visit may be split within 7 days (i.e. half of the visit on Monday and half of the visit on Friday of the same week).**

In the event of early withdrawal, the reason for the withdrawal will be recorded. **Notably, according to the intent-to-treat principal, non-adherence to the trial intervention is not an exclusion criterion for further follow-up.** We encourage all participants to return to the clinical visits and participate in the phone interviews independent of the adherence to the study protocol. When participants express their wish to completely withdraw their involvement in the study, they will be asked to attend a drop-out visit as soon as possible aimed to safety considerations (general health, adverse events, capsules return) when the withdrawal takes place outside the scheduled clinical visit. Participants will be still encouraged to attend the last follow up clinical visit scheduled at 3 years after enrollment. If not willing to attend such last visit, they will be asked for consent to be contacted on the phone at 3 years after the enrollment date. The study staff in each center will be responsible to collect the circumstances of each withdrawal in a standard drop-out

form. For quality purposes, participants will be contacted retrospectively to invite them to the final 36-month visit. In the case of early withdrawal, all study material collected up to the time point of withdrawal will be analyzed, including all blood samples. We will ask specifically to consent to that: both in the case of study drop-out and consent withdrawal, all study material collected up that point can be used for analyses, including blood samples. All participants will be required to sign the consent.

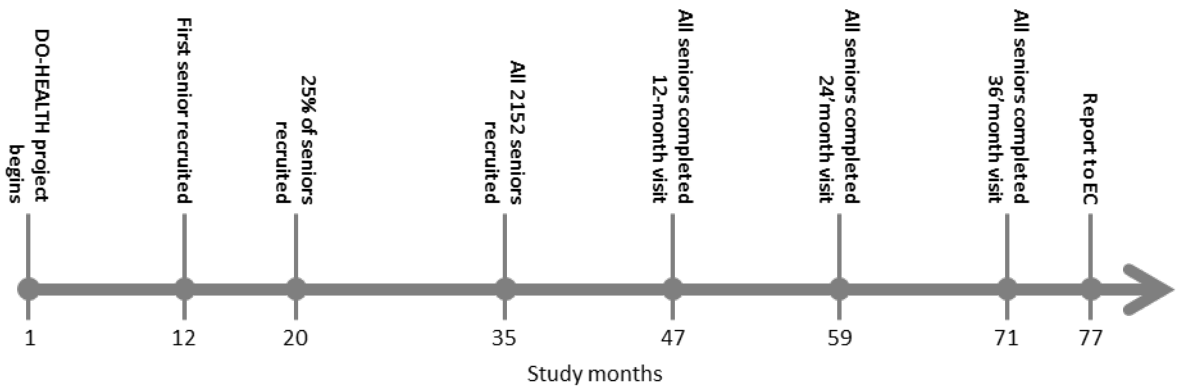
Following is the list of reasons that could potentially lead to early drop-out from the study:

- Adverse events
- Abnormal test results that resulted in physicians' advice to discontinue participation in the study
- Gross protocol violations
- Voluntary consent withdrawal
- Death

7.12. Timeline

DO-HEALTH clinical trial preparation started in January 2012, and the project is designed to last a total of 74 months from beginning to end. The first 11 months of the project will be used for preparation of the trial manuals of operations, developing and testing data entry platform for transfer and storage of study data, developing the video and paper materials for home-based exercise intervention and control exercise program, translating all study materials into the three DO-HEALTH official languages (German, French, and Portuguese), designing study manuals and performing staff training sessions. Further, ethical approval will be achieved in all countries and centers, and the trial will be registered in the international trial registry. **Screening** will start once ethical approval is achieved and as early as month 11. The **recruitment** period will begin in month 12 of the trial and is expected to last for 20 months (until Month 32). 25% of the study population will be recruited by the end of month 20 as a requirement for each center. Due to differences in regulatory and approval procedures in different countries, some centers may start recruitment later than month 12. The latest foreseen deadline to start recruitment is month 14 which shortens the recruitment period for these centers to 18 months. **Centers that have not achieved 25% of their recruitment by month 20 will be closed for further recruitment** (see section 7.8. Recruitment Goals and Recruitment Centers). All study participants are expected to complete their 12-month, 24-month, and 36-month clinical visits by month 47, 59, and 71 of the study respectively. The last 6 months will be required for closing the data bank, data analysis and preparation of final report to the European Commission, competent Authorities and IECs. DO-HEALTH timeline is presented in Figure 6.

Figure 6. DO-HEALTH Project timeline.



8. Study Treatments

The study capsules will be produced by DSM Nutritional Products Ltd., Switzerland (DSM).

Seniors will receive 2 gel capsules per day to keep the size small for easy swallowing. The capsules will be identical in size, looks, taste, and weight. Based on randomization to the 8 study arms, the capsules will include vitamin D and/or omega-3 fats and/or placebo. Vitamin D and omega-3 fats will be mixed in the same capsule for groups 5 and 8:

	Vitamin D 3	Marine Omega-3 fatty acids
Group 1 (n = 269)	Placebo	Placebo
Group 2 (n = 269)	Vitamin D3 (2000 IU/d)	Placebo
Group 3 (n = 269)	Placebo	Omega-3 fats (EPA+DHA, 1 g/d)
Group 4 (n = 269)	Placebo	Placebo
Group 5 (n = 269)	Vitamin D3 (2000 IU/d)	Omega-3 fats (EPA+DHA, 1 g/d)
Group 6 (n = 269)	Vitamin D3 (2000 IU/d)	Placebo
Group 7 (n = 269)	Placebo	Omega-3 fats (EPA+DHA, 1 g/d)
Group 8 (n = 269)	Vitamin D3 (2000 IU/d)	Omega-3 fats (EPA+DHA, 1 g/d)

8.1. Vitamin D_3

8.1.1. **Product description**

Each active vitamin D capsule will contain 1000 IU of Vitamin D_3. It is an oily liquid consisting of crystalline Vitamin D_3 (cholecalciferol) in medium chain triglycerides, stabilized with dl-α-tocopherol (vitamin E, 2.5 promille).

8.1.2. **Dose justification**

We chose **2000 IU Vitamin D per day** as *the treatment dose for vitamin D* based on the data from the DO-HEALTH pilot trial (7) where 2000 IU vitamin D compared to 800 IU vitamin D per day reduced hospital readmission by 39%, fall-related injury by 60%, and severe infections by 90%. Further, 2000 IU vitamin D raised 25-hydroxyvitamin 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 (7). Moreover, in our pooled participant-level analysis of 11 double-blind randomized trials fracture reduction was only significant at the highest intake quartile of vitamin D (792-2000 IU/day)(8). In November 2010 the

Institute of Medicine increased the safe upper intake limit for vitamin D from 2000 to 4000 IU per day (51). The safety of 2000 IU/d of vitamin D is further supported by our benefit-risk analysis where a safe upper intake level of 10,000 IU of vitamin D per day was estimated (1). As all DO-HEALTH participants, according to current guidelines (10), are allowed to an additional vitamin D intake of 800 IU per day, the maximum total intake of vitamin D will be 2800 IU per day in the treatment group and 800 IU per day in the control group, which is below the safe upper intake as defined by the Institute of Medicine (51).

We have not included calcium supplementation as a component of the intervention, as at the higher dose of vitamin D intake, additional high dose calcium supplementation (1000+ mg) was not beneficial for fracture reduction in a pilot study of DO-HEALTH pooling the source data of 11 double-blind RCTs (8); and calcium supplements given without vitamin D may increase the risk of hip fracture(20), the of risk myocardial infarction (21) and the risk of kidney stones (22). In the participant information, we encourage to cover calcium needs sufficiently from dietary sources. A personal intake of calcium supplements will be allowed in DO-HEALTH up to a dose of 500 mg per day.

8.2. Omega-3 Fatty Acids

8.2.1. **Product description**

Each active omega-3 capsule will contain 500 mg of eicosapentaenoic acid (EPA) and docosahexanoic asid (DHA) in a ratio of 1:2. The formulation is an oily liquid containing at least 75% n-3 polyunsaturated fatty acids in the form of ethyl esters predominantly as EPA and DHA. It is stabilized with mixed tocopherols and ascorbyl palmitate. Rosemary extract is used as a processing aid.

8.2.2. **Dose justification**

We chose **1 gram omega-3 fatty acids per day** as *the treatment dose of omega-3 fatty acids as this is* recommended by the American Heart Association for cardio-protection and was beneficial in one secondary prevention trial (11). Because the optimal ratio of EPA to DHA is unknown (12, 13) we have selected a 1:2 ratio of EPA (eicosapentaenoic acid) to DHA (docosahexaenoic acid). Health authorities recommend 400 mg to 1 g per day for cardioprotection. A total dose of 850 mg/d was used in the GISSI-Prevenzione Trial (EPA to DHA ratio, 1:2) (11) and AREDS (EPA to DHA ratio, 2:1)(14), whilst a dose of 1.8 g/d of EPA was used in JELIS (15). Given that the average intake of EPA+DHA is 100 to 350 mg/d in many parts of Europe (16, 17), the proposed intervention of 1 g/d would be expected to increase the average participant's omega-3 intake by a factor of 3 to 10. Health risks associated with fish oil are believed to be minimal and marine omega-3 fatty acid doses of up to 3 g/d are generally recognized as safe. Although omega-3 fatty acids have potential antithrombotic effects, systematic reviews of data from small, short-term trials suggest that omega-3 fatty acid supplements do not increase the risk of clinically significant bleeding at doses of up to 4 g/d, even in combination with anticoagulant interventions such as aspirin or warfarin (18, 19).

High oleic sunflower oil will be used as placebo for both Vitamin D_3 and omega-3 fatty acids. High oleic sunflower oil is cold pressed from organic sunflower kernels, refined and deodorized. The appearance is clear, light yellow and liquid.

8.3. Manufacturing of study capsules

DO-HEALTH study capsules will be soft gel capsules containing Vitamin D3 and/ or Omega 3 fatty acid and/ or placebo (high oleic sunflower oil) – application as a dietary supplement.

Capsule manufacturing

DSM Nutritional Products will provide the active ingredients Vitamin D3 (oily liquid for pharmaceutical and food preparations) and Omega-3 fatty acid (marine algae omega-3 fats EPA and DHA as nutritional oil). Both are nutrients used as dietary supplements, and in the preparation of fortified foods. In addition, Omega-3 fatty acids are regularly used as food ingredients in infant formulations.

Vitamin D3 and omega-3 fatty acids will be sent from DSM to the manufacturer (Swisscaps/ Anova), which is certified for food GMP (good manufacturing processes for food grade) and has the facilities for manufacturing softgel capsules. Anova has introduced an HACCP (Hazard Analysis and Critical Control Points) concept for the production of food/ dietary supplements on the basis of guidelines from the Codex Alimentarius and the BRC (British Retail Consortium). In addition, Anova has ISO 14001 and ISO 9001 certification. Anova can guarantee a consistently high level of quality as a result of their SOP system. All production sites are regularly monitored by the relevant supervisory authorities including national organizations as well as a variety of international bodies (http://www.aenova.de/en/healthcare-en.html).

Capsule Analytics

The DSM ISO certified analytical services laboratory will perform from each batch of produced softgel capsules analytical testing for the correct content of vitamin D3 and/ or omega-3 fatty acids based on validated methods. Furthermore, the analytical services laboratory will evaluate the stability of vitamin D3 and/ or omega-3 fatty acids over the course of 3 years at 25°C in the soft gel capsules. Stability tests will be performed at 25°C at 0, 1, 2, 3, 6, 9, 12, 18, 24, 36 months, at 40°C at 1, 2, 3, 6 months and at 4°C for emergency only. Capsules will be released by DSM QA.

The capsules will only be sent to the centers after signing the Material Transfer Agreement by each centre (part of the Consortium Agreement). The vitamin D3 and omega-3 fatty acids capsules produced for DO-HEALTH are NOT intended to be marketed. The clinical centers will use the capsules only for the conduct of DO-HEALTH. Under no circumstances the capsules will be administered to humans others than those being involved in this study. The capsules will be used only for a limited time period and upon completion of the study any unused capsules will be destroyed.

The appendix outlines the history and arguments of vitamin D and omega-3 fats supplementation as a dietary supplement.

8.4. Home-Based Physical Exercise Program

8.4.1. Program description

A motivational and animated video will be developed to instruct the program as the main exercise intervention in DO-HEALTH. Seniors who want to step up the intensity of the program will be suggested to repeat the whole program, thus increasing the training time. For the exercise control group, a similar animated video will be designed including range of motion exercises (Flexibility exercise program), which were 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 will be developed.

The program will be instructed once during the baseline visit by an "instruction physiotherapist" not involved in the outcome assessment of DO-HEALTH. The aim of this instruction is to make sure that all exercises are performed safely, and correctly adapted to the functional level of the participant. The physiotherapist who instructs the program during the baseline visit will also explain how the video is used and will go over the paper version so that all seniors understand how they can perform the exercise program at home. The physiotherapist also instructs the participant on the training strategy. If during the trial any problems related to the program occur, the participant may call the recruitment centre and the "instruction physiotherapist" will call back the participant to help with the problem. If there is pain with exercise, the general instruction is to reduce repetitions or take a 1 week break.

Exercise intervention (Strength exercise) program will consist of the following exercises:

1. Sit-to-stand (quadriceps strength training)
2. One-leg stance (hip muscle strength training plus balance training)
3. Pull Backs against elastic resistance (seated position)
4. External shoulder rotation against elastic resistance (seated position)
5. Steps

Control exercise (Flexibility exercise) program will consist of the following exercises:

1. Hip and knee mobility (seated position)
2. Hip mobility (standing position)
3. Trunk and chest mobility (seated position)
4. Shoulder mobility (seated position)
5. Ankle mobility (standing position)

8.4.2. Program justification

This simple exercise program was validated in the DO-HEALTH pilot trial (7). Results of the trial suggest that the program is successful in reducing the rate of falling by 25% (significant) and the rate of fracture by 56% in senior subjects after hip fracture (95% CI: [−82% to 9%]; *P* =.08; pilot study was not powered for fracture reduction) in the first year after the fracture (7). Seniors 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). Adherence to the program was 69% after 12 months without any supervision (7).

8.4.3. Motivation and adherence considerations

In the DO-HEALTH pilot trial (7) benefits on strength and function were achieved if the program was performed at least once a week (see section above). As part of the intervention and later broad applicability of the program, an SME partner of DO-HEALTH (BG) will design an innovative and motivational instruction video for seniors to follow the exercise program, and an instruction booklet (paper version of the program). For the exercise control group, a similar animated video and booklet will be designed including range of motion exercises (control exercise). Participants will be asked to do the exercise programs (intervention or control) 3 times per week for 30 minutes. The study staff and participants will be informed that both interventions are beneficial and every in-person contact (every 3 month) will be used as an opportunity to motivate participants in their adherence to the "the exercise intervention" without specifying which program. The outcome staff will remain blinded.

8.5. Blinding techniques

8.5.1. Blinding techniques

Study capsules and exercise kits will be blinded by a central randomization centre. The producer of the study capsules (DSM Nutritional Products Ltd., Switzerland) will send the study capsule bottles, each one containing the monthly capsule supply, to the randomization centre, which will package 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) will be labeled by the randomization centre with another unique randomization code. The web-based randomization service, developed and supported by the DO-HEALTH software partner (FDS), will store the information linking the randomization codes to the actual content of the study capsule kits and exercise kits (there will be no access to this code till the data set is frozen and the study is unblinded). Only in an emergency this information will accessed and made available to authorized users (see next paragraphs).

The central randomization centre and/ or local PIs will be unblinded only in case of an emergency of a participant. In no case will the information be available to other study staff members or the DO-HEALTH central management team. Thus, study interventions will be double-blinded in DO-HEALTH.

Sets of study capsules and exercise kits will be sent to the recruitment centers with the individual coding. Externally, both study capsule kits and exercise kits will appear identical, except for the randomization code on the labels. All study staff members at each trial site will be blinded to treatments, with the exception of the "instruction physiotherapist" who will instructs the program at baseline (unblinding only with respect to the type of exercise); therefore, the physiotherapist will not be part of the assessment team to ensure that blinding is respected.

Once a senior participant is enrolled, the recruitment centre logs into the web-based system to randomize the participant. Randomization will be stratified by centre, prior low trauma fall, gender, and age (see randomization procedures page 85). The software will provide one randomization code for the study capsule kit and a second for the exercise kit. Thus, the study staff will pick the correct treatment kit and exercise packet already stored at the centre, and will be informed how balanced their recruitment is with respect to the stratification variables.

8.5.2. Breaking of the code

In an emergency, the central randomization centre will break the code using the randomization service software. The study sponsor and coordinator (Prof. Bischoff-Ferrari, University of Zurich) will be notified about all code breaks via this software. At the same time, the local recruitment centre PI will inform the DO-HEALTH Monitor regarding all details and a report is prepared for the Data Safety and Monitoring Board. The code break will also be reported in the subject specific Case Report Form and events leading to the emergency breaking will be recorded in the Serious Adverse Event (SAE) report form.

8.6. Administration of study treatments

8.6.1. Dose, frequency, and route of administration

Participants will be instructed to take orally 2 study capsules – preferentially with dinner - or - when participants take all of their interventions (breakfast or lunch or even at bedtime). To reduce the risk of unpleasant taste (and therefore risk of unblinding) from the study capsules that include omega-3 fats, we instruct participants to take the capsules with dinner (or the preferred meal) and a cold drink (water or juice). To further reduce the potential after taste, capsules will be coated so that they only dissolve in the intestine. All participants are asked to develop a routine that allow optimal adherence. This routine is documented by the study staff.

Participants will also be asked to perform the home exercise program for 30 minutes 3 times a week following instructional video or paper manual provided to them in the individual treatment kits (and as instructed by the "instruction physiotherapist"). Any problems regarding the interventions may be brought to the attention of the respective recruitment centre at any in person contact (the study staff will ask about adherence to the interventions at every 3-month in person contact – see section below) or in-between the regular follow-up interviews. For problems regarding the study intervention, the study MD of the respective centre will contact the participant. For problems regarding the exercise intervention, the respective centre "instruction physiotherapist" will contact the participant. The goal is to support the participant in their adherence to the interventions.

8.6.2. Adherence assessment

Adherence to study intervention will be monitored at each in-person contact (3-monthly phone calls and clinical visits at 12, 24, and 36 months) by participant self-report, also investigating the reasons why or why not participants adhere to the study intervention and/ or any difficulties encountered to follow the intervention regimen. Participants will be asked to start a new bottle after they have finished the previous one and to keep all used and partially used bottles. They will then be asked to bring all used, partially used, full bottles of study capsules to the clinical visits at 12, 24, and 36 months for pill counts. Further, we will measure blood levels of 25(OH)D and PUFA in all participants at baseline, 12, 24, and 36 months to assess adherence to the study intervention.

Adherence to the exercise program will be monitored at each in-person contact (3-montly phone calls and clinical visits at 12, 24, and 36 months) by participant self-report, also investigating the reasons why or why not participants adhere to the exercise program and/ or any difficulties encountered to follow the program without asking about which exercise program is being followed by the participant (intervention = Strength/control = Flexibility). If any of the exercises cause problems, the assessment staff will ask the "instruction physiotherapist", who is unblinded to the type of exercise, to contact the participant.

In addition, participants' **diaries** will be reviewed to assess adherence to the exercise program. If there is discrepancy between the dairy and the in-person contact information, the in-person contact information will be used. Based on prior experience, diaries are often not well kept in the target population, but may be useful in some cases (i.e. health care utilization, fall date). Adherence will be supported by motivational strategies at each in person contact (every 3 months).

8.7. Handling of Study Interventions

8.7.1. **Supply, labeling, storage, and distribution**

Four study capsule batches will be produced based on expiry date. Production batch will also include an additional amount of study capsules required for stability control and some additional amount needed in case bottles are lost by a participant.

Study capsules will be packaged in opaque plastic bottles containing 62 capsules each. The permanent labels that will be added by the central DO-HEALTH randomization centre will be printed according to Annex 13 regulation. In particular, they will report the **unique study number** (treatment kit number) and the following information:

DO-HEALTH study capsules (vitamin D_3 1000 IU / omega-3 fatty acids 500 mg / Placebo)
Please take two soft gel capsules daily with dinner. Store in a dry place at room temperature (15 – 25°C) and protect from light.
Primary Investigator and Sponsor: University Hospital of Zurich (Prof. H.A. Bischoff-Ferrari, Rämistrasse 100, CH-8091 Zurich, Tel.: +41 44 255 26 99)
Batch Nr.
Expiry Date

In addition, the sentences "For clinical trial use only", "Keep out of reach of children" and "return all bottles, whether empty or containing unused capsules, to your study doctor" will be printed on the outer box and/or on the individual bottle labels.

DO-HEALTH treatment kit: The first individual study kit (diet supplement with vitamin D/and or omega-3 / placebo) will contain 12 bottles – yearly supply for individual participant – and will be packaged in a carton box. In addition, there will be an exercise kit with the respective exercise video/ paper version. The study kits (study capsules/ exercise program) as described above will be labeled and shipped by the central randomization centre to the study centre where they will be stored in a dry place at room temperature (15 – 25°C). Each recruitment centre will receive a certain number of treatment kits (capsules and exercise video/booklet) according to its recruitment goal (i.e. the University of Geneva recruitment centre will receive 200 treatment kits for both the capsules and the exercise kit bottles and as they will recruit 200 seniors in DO-HEALTH). In addition, fresh replacement/ back-up kits will also be provided to centers at each new batch shipment. Quality control regarding stability testing will be performed in detail by the manufacturer of the study capsules (DSM Nutritional Products) for each batch. Yearly treatment kits of the study capsules + exercise kits will be sent from the central randomization centre to the recruitment centre after the first kit set was assigned. To perform either exercise program all participants will receive an elastic band to take home.

8.7.2. Study interventions (diet supplement) accountability

Each study centre will keep the Dispensing and Return Logs to account for all study capsules dispensed to and returned by the participants. The logs will contain the intervention code on the bottle and the associated subject ID and randomization code of the participant, date and amount of the study capsules dispensed to the participant, and date and amount of the capsules returned by them. Each participant will be given one-year supply of study capsules until the next clinical visit with instructions to bring unused, partially used, and empty bottles of study capsules with them to the next clinical visit. Upon return of the capsules, a pill count will be performed and documented in the participant file. Then the unused study bottles will be labeled with a cross. When required, study capsules dispensation or unused capsules return will take place outside the clinical visits by authorized staff in each center.

Unused capsules are destroyed only after confirmation by the sponsor. Each study centre will keep the capsule destruction log containing the date and amount of study intervention destroyed. A copy of the log will be sent to the central DO-HEALTH randomization centre and the DO-HEALTH coordination centre at the University of Zurich.

8.8. Concomitant Medication

- Before inclusion: if a volunteer is treated on a regular basis with one or more of the drugs listed below, it is in the responsibility of the study MD (investigator) to decide if the participant is eligible due to the exclusion criterion "… or other serious illness that would preclude participation".
- During the trial: if a participant has to be treated on a regular basis and over a longer period of time with one or more of the drugs listed below, the study MD (investigator) has to decide if the safety of the participant is still warranted.

To make these decisions the Investigators Brochure serves as a reference. In addition the evaluation of the treating physician may be helpful and it is always possible to contact the coordinating study team at Zurich and/or the Coordinator and Sponsor (Prof. H. Bischoff-Ferrari) to help.

List of concomitant medication:

- Phenytoin, Barbiturates
  Are antiepileptic drugs and can cause severe vitamin D deficiency (decreases the 25-hydroxylation in liver cells) for which reason a volunteer taking at least one of these active pharmaceutical ingredients cannot be included (exclusion criterion "Epilepsy and/or use of anti-epileptic drugs").
  If a treatment has to be started during the trial a safety assessment has to be done by the study MD and maybe the additional intake of 800IU/d Vitamin D has to recommended and/or the 25OH-hydroxyvitamin D level has to be monitored (not by the study team – unblinding!).
- Thiazide
  Volunteers taking this active pharmaceutical ingredient can be included.
  A long-term therapy can lead to hypercalcaemia, therefore a safety assessment has to be done and the monitoring of the serum calcium level is recommended either by the study MD or by the general practitioner.
- Glucocorticoids
  Volunteers taking this active pharmaceutical ingredient can be included.

A long-term therapy can lead to a disorder of the calcium metabolism, therefore a safety assessment has to be done and maybe the additional intake of 800IU/d Vitamin D has to recommended and/or the 25OH-hydroxyvitamin D level has to be monitored (not by the study team – unblinding!).

- Cardiac glycosides
  Volunteers taking this active pharmaceutical ingredient can be included.
  A concomitant hypercalcaemia increases the toxicity (arrhythmia) of cardiac glycosides, therefore a safety assessment has to be done and the monitoring of the serum calcium level is recommended either by the study MD or by the general practitioner.

9. Study Implementation

9.1. Screening Telephone Interview

Purpose

1. To collect basic demographic information: name, age, mailing address
2. To determine eligibility for baseline visit
3. To schedule baseline visit

The newspaper advertisement will include some information on the inclusion criteria and the purpose of the study plus a phone hotline for the local recruitment centre (see advertisement below).

Advertisement DO-HEALTH recruitment:

DO-HEALTH – A study to support healthy Ageing

Vitamin D3 – Omega3 – Home Exercise – Healthy Ageing and Longevity Trial

Are you 70 years or older, mobile and living independently at home?

If so, we invite you to participate in a **clinical study** with **Vitamin D3-** and **Omega 3-fatty acid supplements (marine algae)** and a **home exercise program.**

Our aim is to investigate the role of these simple strategies in the prevention of chronic disease at older age. In particular, we are interested in the **prevention** of fractures and falls, functional decline, high blood pressure, cognitive decline and pain from osteoarthritis.

The study will take place at XXXX (e.g. the **Centre on Aging and Mobility** of **Zurich University** by the **City Hospital Waid)**. The study duration will be 3 years and during the study we will ask you to attend **4 full-day clinical visits** in our study centre (one per year) and we will call you by phone for a brief interview every 3 months.

Collected data will be kept strictly confidential. Public transportation cost will be reimbursed.

Are you interested? For more information, please call us at **XXXXXXXXX**

Please note that some conditions will not allow you to participate to the study: taking more than 1000 IU vitamin D per day, hemiplegia, severe kidney or liver disease, parathyroid disease, granulomatous disease (i.e. tuberculosis); or in the last 5 years: history of cancer (other than non-melanoma skin cancer), myocardial infarction, angina pectoris, stroke or transient ischemic attack.

When a prospective participant reads the DO-HEALTH information and places a call to the local DO-HEALTH telephone hotline, an *ad hoc* screening interview will be performed or the study nurse will set up a later phone appointment. For the latter instance, a staff member contacts the prospective participant on the scheduled date and conducts the screening.

Telephone screening questionnaire

Inclusion and exclusion criteria	Yes	No
Inclusion criteria		
1. 70 years or older	☐	
2. Community dwelling	☐	
3. Able to walk with or without a walking aid for at least 10 meters and able to get in and out of a chair without help	☐	
4. Able to swallow study intervention	☐	
5. Understanding of spoken and written country language	☐	
Exclusion criteria		
1. History of cancer (except non-melanoma skin cancer) in the last 5 years		☐
2. Cardiovascular conditions (history of MI, stroke, angina, coronary intervention, transient ischemic attack) in the last 5 years		☐
3. Severe renal impairment or dialysis		☐
4. Hemiplegia or other severe gait impairment		☐
5. Severe liver disease		☐
6. History of hypo- / primary hyperparathyroidism		☐
7. History of granulomatous disease		☐
8. Other serious illness		☐
9. Major visual/ hearing impairment		☐
10. Taking more than 1000 IU vitamin D per day in the 3 months prior to enrollment, or unwilling to reduce Vitamin D intake to 800 IU/ day max during the trial i. 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, may be enrolled after a 3-month wash-out period where the maximum daily intake is limited to 800 IU vitamin D. ii. Provision 2: an individual who consumed an average vitamin D dose higher than 2000 IU/day in the 3 months prior to enrollment, may be enrolled after a 6-month wash-out period where the maximum daily intake is limited to 800 IU vitamin D.		☐
10. Unwilling to stop Vitamin D metabolite/ PTH/ Calcitonin treatment		☐
11. Unwilling to limit Ca supplementation to 500 mg/ day during the trial		☐
12. Past or planned participation to other clinical trials		☐
13. Taking omega-3 fat supplement in the 3 months prior to recruitment or unwilling to refrain from use of omega-3 supplements during the trial		☐
14. Living in assisted living situation or in nursing home or with a partner already enrolled in DO-HEALTH		☐
15. Acute fracture in the last 6 weeks *(temporary exclusion)*		☐

16.Epilepsy and/or use of anti-epileptic drugs		☐
17.More than 3 falls in the last month		☐
18.Osteodytrophia deformans (M. Paget, Paget's disease)		☐
19.For study centers in Germany only: persons who are institutionalized / in prison by court order (§40, Abs. 1, Art. 4, "Gesetz über den Verkehr mit Arzneimitteln").		☐

Eligible* for screening/ baseline visit? ☐ yes ☐ no

**The participant is eligible when all boxes are ticked (all inclusion criteria are present and all exclusion criteria are absent)*

Decision about eligibility for baseline visit is made at the end of the interview and all information recorded during the interview, except for name and mailing address, is archived or destroyed depending on the local regulations. Name and mailing address of the prospective participant are used to mail the participant information and the informed consent form to seniors who pass the screening interview and express further interest in the trial. The potential participant is asked to study the participant information plus the written informed consent form for at least 48 hours, call back for any questions and confirm the baseline visit date.

9.2. Baseline Visit

Purpose

1. To answer all additional questions of the study participant
2. To obtain written informed consent for the study
3. To determine full eligibility
4. To document medical history
5. To assess general health of the participant
6. To randomize the participant
7. To obtain baseline measurements
8. To distribute study intervention + exercise kits

Baseline Visit Flow

Procedures and suggested baseline visit flow
Ideally, the baseline visit should be completed in one day, however, a center is allowed to split the baseline visit over two days to streamline the workflow, or prevent the participants from being overwhelmed. Two conditions need to be respected: *- the participant is randomized on the second day* *- the second part of the visit should take place not later than 1 week from the first part*
1. Information, questions, signing of informed consent
2. Demographic information collection
3. Blood draw and urine sample *(the following blood tests will be performed locally: 1. serum calcium; 2. serum creatinine; 3. Simple blood count to assess baseline prevalence of hematologic abnormalities -- not an exclusion criteria**:* (RBC, Hb, WBC, Tc) with erythrocyte indices (MCV etc.), machine differential (Granuloc., lymphocyte) and reticulocytes*: 1+2 are inclusion criteria*

4. Breakfast
5. Mini mental state examination (MMSE)
6. Height and weight measurements
7. Short physical performance battery
8. Review of inclusion/exclusion criteria, serum calcium level and creatinine clearance check
9. If all inclusion criteria are met, perform randomization via automated DO-HEALTH randomization software to initiate enrollment
10. Blood pressure measurement
11. Physical examination by the study MD (including general appearance/condition, heart auscultation, peripheral oedema, hepatojugular reflux, lung percussion, lung auscultation, abdomen auscultation, abdomen palpation, renal angle tenderness, other (e.g. striking tremor, paralysis, tics, adiposity, cachexia) and visual acuity with Landolt rings)
12. Lunch
13. Bone densitometry at centers Zurich, Berlin, Toulouse, Coimbra* OR
    Dual tasking gait variability at center Basel
14. Grip strength and dual tasking gait speed assessment by the blinded "assessment nurse or physiotherapist"
15. Questionnaire assessment (all questionnaires)
16. Distribution of study interventions (1-year supply) by study nurse and instruction of home exercise program by the "instruction physiotherapist" not involved in endpoint measurements and not blinded to the home exercise program
17. First phone interview scheduled in 3 months, next clinical visit scheduled in 1 year. Information about emergency hotline phone number

* Performed at 4 study centers equipped with DXA machines.

Individual study centers may deviate from the order of procedures in the outlined visit structure if it benefits their operations provided the following conditions be fulfilled:

1. The Informed Consent is signed by the participant and the study physician on behalf of the study investigator before data collection starts or any medical procedure including blood draw is administered to the participant.
2. Blood is drawn before the participant breaks the fast.
3. All inclusion/exclusion criteria (including MMSE score, ability to walk 10 meters, serum calcium level, and creatinine clearance) are checked and fulfilled before randomization.
4. Resource and personnel intensive procedures (bone densitometry, questionnaires, etc.) are to be performed on fully enrolled participants only (after all information is available that all inclusion criteria are met).

9.3. Clinical Visits

All clinical visits should be performed within a time frame of +/- 14 days around the ideal date, which is based on the day the participant was randomized.

Purpose

1. To answer any question the participant may have about the study

2. To obtain measurements for primary, secondary, exploratory, and safety endpoints
3. To collect information about health care utilization, falls, fractures, incident infections and any adverse events during the preceding 3-month period – in case of a fall or an infection: fill in the respective detailed protocols
4. To assess general health of the participant and get information on medications/ supplements outside the study protocol
5. To evaluate adherence to the study interventions and the exercise regimens
6. To assess adverse events and side effects of the study interventions and the exercise regimen
7. To assess why and why not the participant is adherent to the study protocol (assessment of comparative effectiveness)
8. To motivate participants to take their study capsules and to do their exercise program 30 minutes 3 times per week
9. To distribute study intervention (at every visit) and exercise kits (at baseline only)

Clinical Visit Flow

	Procedures with suggested 12, 24, 36 month visit flow
Ideally, 12, 24, and 36 month clinical visits should be completed in one day, however, a center is allowed to split the baseline visit over two days to streamline the workflow, or prevent the participants from being overwhelmed. One condition needs to be respected: - the second part of the visit should take place not later than 1 week from the first part	
1.	Blood draw and urine sample *(the following blood safety parameters will be assessed locally: 1. serum calcium; 2. serum creatinine).*
2.	Breakfast
3.	Blood pressure measurement
4.	Height and weight measurements
5.	Physical examination by the study MD (including general appearance/condition, heart auscultation, peripheral oedema, hepatojugular reflux, lung percussion, lung auscultation, abdomen auscultation, abdomen palpation, renal angle tenderness, other (e.g. striking tremor, paralysis, tics, adiposity, cachexia) and visual acuity with Landolt rings); assessment of adverse events and side effects of the study interventions and of the exercise regimen/ change in medications or supplements outside the study protocol
6.	Lunch
7.	Bone densitometry at centers Zurich, Berlin, Toulouse, Coimbra[†] OR Dual tasking gait variability at center Basel
8.	Short physical performance battery, grip strength, and reaction time (repeated sit-to-stand test is part of SPPB) by assessment nurse or assessment physiotherapist
9.	Questionnaire assessment supervised and supported by the study nurse
10.	Distribution of study interventions (1-year supply)[‡]
11.	Reminder of the emergency hotline phone number and schedule of the upcoming telephone interviews and clinical visits (next phone interview is scheduled in 3 months, next clinical visit is scheduled in 1 year)[‡]

[†] Performed at 4 study centers equipped with DXA machines

[‡] Not performed at 36-month visit

9.4. Telephone Contacts

All phone call visits should be performed within a time frame of +/- 14 days around the ideal date, which is based on the day the participant was randomized.

Purpose

1. To answer any question the participant may have about the study
2. To ensure that the participant has the study intervention and all material for the exercise intervention
3. To collect information about healthcare utilization, falls, fractures, incident infections and any adverse events during the preceding 3-month period – in case of a fall or an infection: fill in the respective detailed protocols
4. To assess quality of life with the phone-based EQ5D-3L at month 6, 18, 30
5. To assess adherence to study intervention and the exercise regimen
6. To assess why and why not the participant is adherent to the study protocol (assessment of comparative effectiveness of both the study intervention and the exercise intervention)
7. To motivate participants to take their study capsules and to do their exercise program 30 minutes 3 times per week

9.5. Biological Samples and Laboratory Procedures

At baseline, 12, 24 and 36 month visit, up to 75 ml (up to 50.5 ml for the trial and possibly other 24.5 ml for the bio-bank – the bio-bank requires a separate informed consent) of blood will be drawn from each study participant and processed into aliquots at the recruitment sites. About 7.5 ml will be immediately used on site for safety measurements and blood cell count. The remaining material, from which we expect to collect about 6 ml of serum, 11.5 ml of plasma and about 12 ml of other whole blood preparations – will be frozen and stored for further analyses at Fisher Clinical Services GmbH (Allschwil, Switzerland). Part of this material (about 4 ml of serum, 7 ml of plasma and 4 ml of whole blood) will be used for laboratory analyses performed in DO-HEALTH; if some blood is left after planned analyses have been run, it will be stored for safety reasons (e.g. repetition of an analysis) for up to 10 years after end of study (last patient, last visit). The blood collected for the bio-bank (separate additional consent) will be stored in 0.5-ml, or larger, aliquots in DO-HEALTH biobank at Fisher Clinical Services GmbH (Allschwil, Switzerland) for future biomarker – as well as metabolomic and genomic – analyses, depending on additional funding for up to 30 years after end of study (last patient, last visit).

The total volume of blood will differ by centre due to “inflammatory” and “future metabolomic and genomic” analyses (Zurich will collect a total of 75 ml of blood, Basel 65 ml, and all other centers 55 ml of blood).

All aliquots processed at recruitment centers will be temporarily stored at the recruitment centers at -80°C. The label attached to each aliquot will show participant’s ID, type of blood aliquot (serum, plasma, whole blood). Shipments of aliquots from the recruitment sites to Fisher Clinical Services GmbH will be on a 3-monthly basis in dry ice containers, so that the cold chain is not disrupted, and stored at the central DO-

HEALTH biobank at Fisher Clinical Services GmbH (Allschwil, Switzerland) under the same temperature conditions (-80°C) until analyzed.

Most biomarkers will be quantified in the central DO-HEALTH laboratory at Zurich University Hospital (Institute of Clinical Chemistry) at baseline, 12, 24, and 36 month after all samples for a corresponding time point have been collected. The central laboratory is accredited according to ISO/IEC 17025. All clinical chemistry tests and immunoassays will be provided by Roche Diagnostics and performed on a COBAS8000 auto-analyzer, including an automated 25-hydroxyvitamin D assay. DSM Analytical Research Center will perform 25(OH)D measurements with gold standard LC-APPI-MS/MS methodology and PUFA measurements by a sensitive and selective assay based on liquid chromatography coupled to mass spectrometry detection (LC-MS/MS). DSM Analytical Research Center is accredited according to ISO 9001:2008. Two novel biomarkers – myostatin and sclerostin – will be assessed at Dresden Technical University; other novel markers of inflammation and immunity will be assessed at Max Rubner Institute. All aliquots are stored centrallyat Fisher Clinical Services GmbH (Allschwil, Switzerland) before distribution to the different laboratories. The aliquots for the inflammation and immunity marker analysis will be shipped on a three monthly basis directly to Max Rubner Institute. In each clinical visit, safety markers required (calcium and creatinine clearance) will be analyzed by express tests right after blood collection at each trial site. Only at baseline and to assess blood count abnormalities, such as anemia, a small blood count will be performed locally as well.

The laboratory results structure will be coordinated with the Data Management Team with restricted access to results to avoid potential un-blinding via laboratory results. The handling and labeling of all samples will be anonymous and standardized, and implemented by the Data Management Team of DO-HEALTH. Table 2 summarizes laboratory analyzes planned for DO-HEALTH.

Table 2. Summary of laboratory procedures.

Biomarkers	Analyzing Laboratory	Analysis Procedures
Organ-specific markers: calcium, phosphate, intact PTH, urinary calcium/creatinine ratio (second spot urine), Beta-Crosslaps serum, P1NP, Troponin T, NT-proBNP, homocysteine, CK, cholesterol, HDL-cholesterol, triglycerides, hs-CRP, IL6, AST, ALT, gGT, alkaline phosphatase, bilirubin, fasting glucose, insulin, serum creatinine; serum urea, uric acid; Ions: sodium, potassium, chloride, magnesium; Proteins: total protein, albumin, ferritin, transferrin, TSH, fT4, fT3, cortisol; vitamins: folic acid, vitamin B12, 25(OH)D with automated assay (adherence)	Central DO-HEALTH laboratory, Institute of Clinical Chemistry, University Hospital Zurich (ISO Certified ISO/IEC 17025) Note: Safety biomarkers for trial inclusion (creatinine, calcium) plus baseline blood count at each trial site	All clinical chemistry tests and immunoassays will be provided by ROCHE Diagnostics and performed on a COBAS8000 auto-analyzer
Novel bone markers: myostatin, sclerostin	Technische Universität Dresden	Concentration for sclerostin and myostatin will be determined using a biotinylated detection

Biomarkers	Analyzing Laboratory	Analysis Procedures
		antibody
Markers of inflammation and immunity (assessed in the participants recruited at the University of Zurich and Basel): TNFalpha, IL-10, IL-17, IL-22, CD3, CD4, CD25, CD127	Max Rubner Institut Bundesforschungsinstitut für Ernährung und Lebensmittel Technologie	Biomarkers of inflammation will be measured with ELISA, phenotyping of immune cells will be done by flow cytometry
Adherence markers: 25(OH)D polyunsaturated fatty acids (PUFAs)	Analytical Research Center, DSM (ISO certified ISO 9001:2008)	25(OH)D will be measured by LC-APPI-MS/MS. PUFAs will be measured by liquid chromatography coupled to mass spectrometry detection (LC-MS/MS)

9.5.1. Biological sample storage for genetic and metabolomic analyses (biobank)

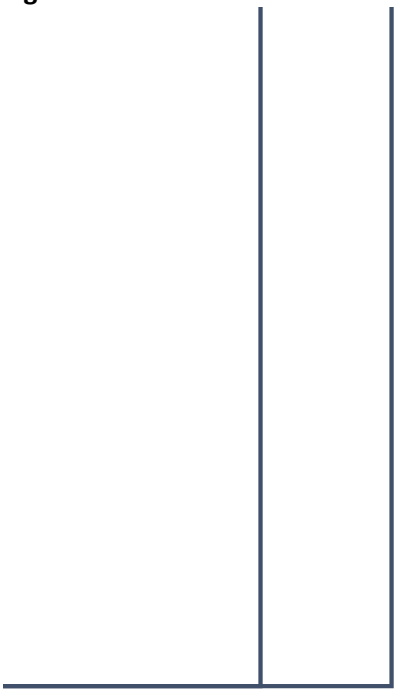
The DO-HEALTH biobank is established for future exploitation of the rich DO-HEALTH data set addressing research questions that require additional bio-analyses. The DO-HEALTH central biobank will be located at Fisher Clinical Services GmbH (Allschwil, Switzerland) and will have a coherent structure with the DO-HEALTH central data bank. To explore whether the response to vitamin D and omega-3 fatty acid supplementation is influenced by genetic factors, genetic association analyses between genetic polymorphisms of relevant genes of the vitamin D and PUFA pathways will be analyzed and correlated with clinical endpoints of DO-HEALTH once funding is secured for these analyses (nutri-genomic analyses). Further, if future funds become available, the biobank will be set up to provide material to assess the metabolomic profile before and after the intervention in DO-HEALTH to further elucidate their impact on senior physiology and multi-organ ageing. Further, additional serum, plasma and urine aliquots will be stored to address future research question (if ancillary grants will become available). All samples stored in the central DO-HEALTH biobank at Fisher Clinical Services GmbH (Allschwil, Switzerland) will be stored by subject ID only to maintain a high level of data security. Participants will be able to choose if releasing the consent for biobank storage or not. Even though c**onsent to the biobank is strongly encouraged to optimize exploitation of DO-HEALTH data collection, it is not allowed to exert any kind of pressure on participants.**

Apart from the metabolomic analyses (which due to logistic reasons will be limited to extra blood samples drawn at the recruitment centre in Zurich), all future analyses performed on the blood samples from the DO-HEALTH biobank will represent the whole DO-HEALTH sample. The biobank of DO-HEALTH will be established for analyses of the complete DO-HEALTH study sample. For future research based on biological samples of the DO-HEALTH biobank, individual recruitment centers cannot request local analyses from their participants only. All future analyses are coordinated by the DO-HEALTH coordinating center responsible for the DO-HEALTH data bank and future exploitation of the biological samples.

10. Data Management

Figure 7 summarizes the data collection and storage system that will be implemented for the DO-HEALTH Project. Red arrows show the flow of data associated with biological specimens and blue arrows represent the flow of all other electronic data within the study. All study centers will be structured identically from the perspective of data collection; the schematic diagram depicts the structure of one study center.

Figure 7. DO-HEALTH data collection and storage system.



10.1. Data Entry and Collection

DO-HEALTH will use an electronic data collection (EDC) system that will be designed for the study by a DO-HEALTH partner (FDS). The system includes the software and hardware for entering, transferring, storing, and accessing the data, as well as for monitoring data collection and controlling and recording access to the data.

Questionnaires and forms will be created, translated into the study languages, reviewed by the PI and the recruitment partners of DO-HEALTH, and implemented in the EDC system. All data collected in the study (participant-reported data, laboratory data, data obtained from physical tests and examinations) will be considered source data, and therefore data will be entered directly into the EDC system during visits/ phone calls by either the participant themselves or an authorized member of the staff. In case of technical difficulties, paper forms will also be available and the information will be entered later into the EDC system as per the following step-wise standard procedure: an authorized company meeting all European

Commission standard requirements for CTs will scan on-site (*i.e.*, at the study sites premises) all the CRFs which were previously completed on paper. An electronic copy (in the form of PDF files of scanned CRFs) will remain at the study site, and an electronic copy will be provided to the Coordination Centre for Clinical Trials (KKS) of the Medical University of Vienna (Austria), in charge of the double data entry into DO-HEALTH database. The CRFs will be coded and will not contain any personal information from the participants. The code will remains only at the local study site. Several platforms will be available for data entry, including electronic tablets such as iPads and standard workstations. The data entry devices will detect and flag problematic data points before the visit is concluded, so that staff can address problems immediately. Upon completion of a visit, the participant may be asked to verify some key pieces of data.

10.2. Transfer and Storage of Data

Throughout the EDC system (on data entry devices, on local site workstations, and in the central databank), data from both forms and biological samples in the central DO-HEALTH biobank will be stored by subject ID, without any personally identifiable data, and access will require an authorized login. The data will also be printed out as human- and machine-readable paper forms and securely stored for the duration of the project.

Study sites will be provided with software enabling safe and secure transfer of the collected data from data entry devices through a site-local workstation and on to the central databank using encrypted protocols. The data will be transferred as soon after the completion of the visit or phone call as possible, usually immediately.

Every 3 months the biological samples stored at the local sites will be sent to the central DO-HEALTH blood bank at Fisher Clinical Services GmbH (Allschwil, Switzerland) for analysis and long-term storage. The data from the analysis of the samples will be added to the central databank. For the analyses performed outside the DO-HEALTH coordinating centre, all samples per visit will be collected first at the coordinating centre, and then sent to the four external laboratories (partners at DSM, Max Rubner Institut Bundesforschungs-institut für Ernährung und Lebensmittel Technologie, Dresden University). The 3 external laboratories will perform the respective analyses and send back the results by Subject ID to the DO-HEALTH data management team at the coordinating centre to include the data in the central DO-HEALTH data base. The material not used for the external analyses will be stored at -80 degrees Celsius at the external laboratories and then sent back to the central DO-HEALTH blood bank Fisher Clinical Services GmbH (Allschwil, Switzerland) for storage. No material will remain at the external laboratories.

All DXA data from the 4 recruitment centers with DXA machines will be collected by the DO-HEALTH data management team at the coordinating centre at the University of Zurich and by the Berlin DXA Assessment Centre. Two dedicated email accounts at the University of Zurich (dexa@do-health.eu) and at Berlin DXA Assessment centre (do-health@charite.de) will be used solely for the purpose of DXA data transfer). DXA measurements will be identified via DO-HEALTH study ID, as Berlin DXA Assessment Centre is a different unit from the recruitment centre in Berlin Charité. After the external assessment by the Berlin unit, all DXA data including vertebral morphometry will be sent back to the data management team at the coordinating centre for central collection and analyses.

DO-HEALTH will store all of its data in the central databank that ensures top security and reliability of the data. The central databank will be backed up daily, and backups will be regularly verified and copied to a secure second physical location.

10.3. Data Quality Control

Step-by-step descriptions of all procedures performed during clinical visits and phone interviews will be documented and followed. The Standard Operating Procedures (SOPs) are mandatory and have to be followed by all personnel involved. To do so, all personnel involved in clinical visits and phone interviews will be appropriately trained and certified before they are allowed to perform data collection.

The EDC software at the study centers will be programmed to detect and flag common types of errors (missing answers, out-of range values, inconsistent information, etc.) at the data entry stage, allowing the personnel to correct the data immediately. If errors are detected, the software will not allow proceeding further unless the error is corrected. In exceptional circumstances this feature may be bypassed by a staff member using a specially designed procedure, and this exception will be flagged in the databank. A procedure will be implemented requiring the study centre personnel to double-check critical data points before the participant leaves the study centre after a clinical visit.

10.4. Data Anonymity and Access to Study Data

All data transferred to the central data base or stored in the databank will be de-personalized; subject ID will remain the only data identifier for the purposes of analysis.

Each site will maintain a table linking subject names and contact information to subject IDs. Access to this table will be limited strictly to staff requiring this information, and no personally identifying information from this table will be entered into the EDC system.

Each individual involved in the management of study data will have a unique user name and password to access the databank. Assess rights for each such individual will be restricted in accordance to his/ her function at the local recruitment site so that exposure to sensitive data is minimized, and access to data will be logged and available for reporting. If a participant is to enter data himself, the electronic form for that subject must be opened by the staff member, and once the subject is given access to the data entry device, leaving the form will require that the staff member re-enter his/ her password.

All data analyses with DO-HEALTH data will be performed centrally at the DO-HEALTH coordination centers by DO-HEALTH data management team to further ensure data control and safety. Partners who collaborate on the analyses are asked to send a pre-defined analysis plan to the data management team. DO-HEALTH data will not be transferred outside the DO-HEALTH coordinating centre, again to ensure data control and safety, as well as high quality statistical analyses support.

10.5. Audit

Regular audits will be run on the central databank, reporting summary statistics (ranges, means, medians, missing values, and exceptional conditions) on key variables. The results will be reviewed by the DO-HEALTH data management team (data programmer and epidemiologist) and the head biostatistician (Prof. E. John Orav, Harvard Medical School) and logged for future reference. Study centers will be notified of identified problems both so that the data can be corrected if possible, and so that the cause of the error can be

identified and avoided in the future. If necessary, additional training will be provided or other actions taken to rectify the situation. All study staff (nurses, MDs, physiotherapists) working on DO-HEALTH will need to be certified by that DO-HEALTH coordinating centre before they work within the DO-HEALTH study.

10.6. Records Retention at the Study Site

According to FDA and EMEA regulations, the investigator(s) participating in this clinical trial will maintain detailed clinical data as required by EU directive 2003/63/EC or for a 15-year period, whichever is the longest, starting from the official study end date communicated by the sponsor to the relevant authorities.

The investigator should not dispose of any records relevant to this study without either (1) written permission from the sponsor or (2) providing an opportunity for the sponsor to collect such records. The investigator, with relevant assistance, will take responsibility for maintaining adequate and accurate electronic or hard copy source documents of all observations and data generated during this study, including the CRF data. Such documentation is subject to inspection by the monitor, the sponsor and relevant regulatory agencies.

Furthermore, if the investigator at a recruitment centre withdraws from the study (i.e.: relocation, retirement), all study related records should be transferred to the DO-HEALTH coordination centre at the University of Zurich for safe archiving.

11. Statistical Considerations

11.1. General Principles and Data Analyses

General principles: All analyses plans will be pre-defined and reviewed by the PI, the DO-HEALTH epidemiologist, and the head biostatistician (Prof. E. John Orav). All necessary procedures for the analyses plans will be coded in SAS statistical language and stored as electronic SAS files in the designated storage. An analysis log-book will be prepared for all analyses to be run for baseline comparisons and when the trial is finished.

All analyses will be performed centrally by the data management team at the DO-HEALTH coordinating centre using SAS v9.2 (or later) statistical software (Copyright© 2002-2003 by SAS Institute Inc., Cary, NC, USA) and supplemented by diagnostic techniques to assess the adequacy of statistical assumptions. Since the study assesses 5 primary endpoints, a Bonferroni adjustment for multiple comparisons will be applied in the statistical analysis so that $p < 0.01$ is required for statistical significance. In analysis for all other endpoints, a p-value of 0.05 (two-sided) will be considered a threshold for statistical significance.

Data analysis: Analyses of treatment effects will be based on the intent-to-treat principle. The first analysis will compare baseline characteristics by randomized treatment assignment to ensure that balance was achieved by the randomization. Characteristics to be examined include known risk factors for the primary endpoints, including age, gender, BMI, smoking, alcohol use, physical activity, comorbidity, baseline blood pressure, baseline cognitive function (MMSE and MoCA score), baseline lower extremity function (SPPB score), prevalence of prior fall, and baseline 25(OH)D and omega-3 fatty acid blood levels. The large sample size, as well as successful balance of known potential confounders, will provide assurance that unmeasured or unknown potential confounders will also be equally distributed across randomized treatment groups.

In this 2×2×2 factorial design, the primary aim is to compare the main effects of **intention-to-treat** with vitamin D, omega-3 fats, and the home exercise program on the 5 primary endpoints. For **incident non-vertebral fractures**, we will use the Cox proportional hazards model to allow for variable follow-up lengths (284) and will estimate the hazard ratio for each intervention using indicators for treatment exposure, controlling for the other two interventions, age, and gender, and competing risks. For the continuously measured primary endpoints the **risk of functional decline**, the **risk of blood pressure increase**, the **risk of cognitive decline**, we will use repeated measurement analyses and will estimate the mean difference for each intervention using indicators for treatment exposure, controlling for the other two interventions, age, and gender, and competing risks. For the **rate of incident infections** endpoint, we assume a Poisson distribution for the number of infections, and will use a Poisson regression analysis to compare the number of infections for each intervention using indicators for treatment exposure, controlling for the other two interventions, age, and gender, and competing risks. Similar analyses will be performed for the secondary, and exploratory endpoints and the biomarker endpoints.

Per protocol analyses: Beyond the intent-to-treat analyses, we will examine the effect of the interventions among adherent individuals. For Vitamin D and omega-3 fatty acids, adherent individuals are defined as

individuals who took 80% of their capsules. For the exercise program, adherent individuals are defined as individuals who performed the exercise program at least once per week in 80% of cases.

Beyond the primary analyses, we will examine **effect modification** by the other randomized interventions, by baseline risk factors, and by time. With respect to **subgroup analyses**, we will test whether vitamin D3 and /or omega-3 fatty acids (EPA+DHA) and /or the simple home exercise program reduce the risk of primary and secondary endpoints differentially by gender, age (70-84; 85+), body mass index, baseline physical activity, baseline serum 25(OH)D levels, baseline PUFA levels, previous fall (last year), previous fracture (last 10 years), FRAX – estimated absolute fracture risk, baseline symptomatic knee OA, and baseline calcium and protein intake (diet + supplements).

We will also **compare the incidence of potential side effects** in the active *vs.* control groups for each treatment, including the incidence hypercalcemia and kidney stones with vitamin D assignment (Women's Health Initiative calcium-vitamin D findings (22)) and the incidence of GI symptoms, skin abnormalities, and bleeding with omega-3 fats assignment (JELIS findings (15)).

We will also **assess the comparative effectiveness** for each treatment comparing the adherence between groups and reasons why and why not seniors were adherent to the 3 treatments.

The DO-HEALTH evidence will be used to develop a comprehensive disease and **health economic model** for the 3 interventions combining prospectively collected participant-reported resource utilization and utility data, and modeling downstream endpoints on a country-specific basis.

11.2. Power and Sample Size

We will enroll 2152 seniors; this sample size was chosen to guarantee sufficient power for all primary and the most critical secondary endpoints. Based on prior experience, we expect that 68% will have completed the entire 3-year study. However, the 32% of subjects who are expected to drop out early will still provide partial study data since our analyses will be based on the intention-to-treat principle. For the power calculations below, we have assumed the effective sample size to be 1807 subjects, representing full follow-up data on 68% of the 2152 enrolled seniors (i.e., n = 1463 = 0.68 × 2152), plus an average of half follow-up on the 689 of subjects who are expected to withdraw early (i.e., n = 344 = 0.5 × 0.32 × 2152).

The trial has five primary outcomes and we use Bonferroni adjustment for multiple comparisons so that p < 0.01 is required for statistical significance. The five outcomes will be the rate of any non-vertebral fractures (rate comparison), the Short Physical Performance Battery (continuous), blood pressure (continuous), cognitive decline (continuous), number of infections (Poisson count).

Also, we have tried to increase the efficiency of this study by using a factorial design, under the assumption that vitamin D, omega-3 fatty acids, and the simple home-based exercise program have distinct mechanisms of action and therefore will have an additive effect on our outcomes. We will check this assumption in our analyses by including interaction terms between the three treatments in our initial analysis models and testing for effect modification. If, as we anticipate, there is no significant effect modification, then our power for testing the effect of each intervention will be based on sample sizes of 903 versus 903 (i.e., the effective sample size of 1807 will be divided into the 903 who were randomized to a given intervention,

compared to the other half who did not receive that intervention). Based on our pilot study, we have found no effect modification between vitamin D and exercise and we assume that none will exist in this trial. If, however, we do find significant effect modification between vitamin D and omega-3 fatty acids, then the pure effect of either vitamin D or omega-3 must be measured by comparing the 451 seniors (i.e., one-quarter of the effective sample size of 1807) who received that intervention to the 451 participants who received neither intervention. Our power calculations below show the power of our study for both of these possible scenarios.

For our most critical analysis, assuming that vitamin D has the same effectiveness as in our pilot trial and that we have 3 years of treatment and follow-up in 68% of participants and partial follow-up in the other 32%, we will have 99% power to detect a significant difference in the risk of any non-vertebral fracture. This calculation assumes enrolment of 2152 seniors and no effect modification between vitamin D, omega-3 fatty acids, and the home exercise program. If, however, there is effect modification between vitamin D and omega-3 fatty acids, then we will still have at least 80% power to detect the effect of any of the three interventions. We believe that there will be no interaction with exercise based on out pilot trial (7).

11.2.1. **Power considerations for primary endpoints**

Bone: *significant difference for the risk of any non-vertebral fracture (including hip fractures)*

The expected incidence rate of any non-vertebral fracture in DO-HEALTH is 14% (IR = 0.14) and is based on the placebo groups of all double-blind trials that enrolled individuals age 70+ from our meta-analysis of individual participant data pooled from 11 double-blind RCTs of vitamin D and fracture prevention (in press New England Journal of Medicine) – vitamin D dose finding pilot project for DO-HEALTH (8), while the effect size is based on the pilot trial to DO-HEALTH where we documented a 52% reduction of any non-vertebral fractures (p-value = 0.11) comparing 2000 IU vitamin D to 800 IU vitamin D in 173 seniors over a 12 month follow-up (7). With the criterion for significance set at $\alpha = 0.01$, and a 2-tailed test interpretation, effective sample sizes of 903 and 903 for the main effect of vitamin D will have power of 99% to yield a statistically significant result. This computation assumes that the difference in proportions is 0.073 (specifically, 0.14 vs. 0.067). Under the same assumptions, but smaller effective sample sizes of 451 and 451 due to the presence of effect modification between vitamin D and omega-3, we would still have 80% power to detect the pure main effect of vitamin D of the same magnitude.

Muscle: *significant difference in the mean SPPB score as a measure of lower extremity function in seniors*

The Short Physical Performance Battery (SPPB) 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 (252). The SPPB is a brief performance-based test that includes walking speed, repeated chair stands, and a balance test. Its three components are each scored 0 to 4, with 4 indicating the highest level of performance, and are summed up to yield an overall score. Based on the Lifestyle Interventions and Independence for Elders Pilot (LIFE-P) study the mean SPPB score is 7.52 (SD = 1.42) in community-dwelling seniors age 70 to 89 (285). A minimal meaningful change for the SPPB was found to be 0.4 – 1.5 points. Based on this data and assuming a minimal change of 0.4 points in the SPPB due on the interventions, a criterion for significance $\alpha = 0.01$, and a 2-tailed test, which means that an effect in either direction will be interpreted, effective sample sizes of 903 and 903 for the main effects are sufficient to yield a power of 99%. This computation assumes that the

mean difference is 0.40 (corresponding to means of 7.52 versus 7.12) and the common within-group standard deviation is 1.42. This effect was selected as the minimal clinically significant effect. It is also assumed that this effect size is reasonable, in the sense that an effect of this magnitude could be anticipated in this field of research. If effect modification between vitamin D and omega-3 fatty acids is found for the SPPB, then a comparison of the 451 participants who received a single intervention compared to the 451 participants who received neither intervention would still provide 97% power to detect the same minimal difference of 0.40 at $\alpha = 0.01$.

Cardiovascular: *significant difference in mean blood pressure*

Based on epidemiologic data, we assume a mean systolic blood pressure of 158 mmHg (SD = 24.5 mmHg) in seniors with a mean age of 75 years. Previous trials with 800 IU vitamin D or UVB radiation documented an effect size of 6 mmHg (58, 59), which we confirmed in a pilot study to DO-HEALTH comparing postmenopausal women receiving 800 IU vitamin D to postmenopausal women receiving 20 µg of 25-hydroxyvitaminD(23), which is about equivalent to 2000 IU vitamin D.

Thus, with a criterion for significance set at $\alpha = 0.01$, a 2-tailed test, and effective sample sizes of 903 and 903 for the main effect of vitamin D, the study will have power of 99% to detect a statistically significant result. This computation assumes that the mean difference is 6.0 mmHg (corresponding to means of 158.0 vs. 152.0 mmHg) and the common within-group standard deviation is 24.5 mmHg. If there is effect modification between vitamin D and omega-3, then the smaller sample sizes of 451 and 451 must be used to isolate the pure effect of vitamin D, but the power remains sufficiently high at 82%.

Based on epidemiologic data, we assume a mean diastolic blood pressure of 90 mmHg and a standard deviation SD = 12.4 mmHg in seniors with a mean age of 75 years. Previous trials with 800 IU vitamin D or UVB radiation documented an effect size of 2 to 6 mmHg, which we confirmed in a pilot study to DO-HEALTH comparing postmenopausal women receiving 800 IU vitamin D to postmenopausal women receiving 20 µg of 25-hydroxyvitamin D (23), which is about equivalent to 2000 IU vitamin D.

Thus, with a criterion for significance set at $\alpha = 0.01$, a 2-tailed test, and effective sample sizes of 903 and 903 for the main effect of vitamin D, the study will have power of 99% to yield a statistically significant result. This computation assumes that the mean difference is 3.0 mmHg (corresponding to means of 90 vs. 87 mmHg) and the common within-group standard deviation SD = 12.4 mmHg. If there is effect modification between vitamin D and omega-3 fatty acids, then the smaller sample sizes of 451 and 451 must be used to isolate the pure effect of vitamin D, but the power remains sufficiently high at 86%.

Brain: *significant difference in mean cognitive function*

Cognitive function will be assessed with Montreal Cognitive Assessment tool (MoCA). Being a relatively new tool, MoCA has been validated in several studies against the Mini Mental State Examination (MMSE) (253-257). The two test were found to be moderately to strongly correlated ($r = 0.53 – 0.87$) and MoCA was shown to be more sensitive than MMSE with respect to mild cognitive impairment (253-256). Comparative studies that measured (256) the same senior populations with both MMSE and MoCA report mean MoCA scores 25.2 – 25.9 for the seniors with normal cognitive function (MMSE scores 27.7 – 28.6). Seniors with mildly impaired cognitive function (mean MMSE scores 25.3 – 26.8) had average MoCA scores of 20.4 – 20.5

(256, 257). MoCA scores had slightly larger standard deviations (1.8 – 3.8) compared to those of MMSE scores (1.6 – 3.1).

Zurich disability prevention trial (pilot trial to test recruitment potential and feasibility of DO-HEALTH (258)) used MMSE to assess cognitive function. Study participants were seniors who had a fall during a year prior to recruitment and MMSE score 26 or higher. In this trial baseline mean MMSE score was 28.6 with a maximal standard deviation of 1.0 (n = 200). Comparing seniors with 25(OH)D levels of less than 25 nmol/l to those with 75 nmol/l, their MMSE score decreased by 0.7 points per year over a mean follow-up of 5.2 years. Based on these findings and the fact that MoCA scores tend to decrease faster than do MMSE scores as cognitive function deteriorates, we expect with treatment in DO-HEALTH a conservative 0.7-point difference in MoCA scores in 3 years of follow-up.

In DO-HEALTH, the inclusion criteria for the MMSE will be at least 24. With a criterion for significance set at $\alpha = 0.01$, and a 2-tailed test interpretation, effective sample sizes of 903 and 903 for the main effect of each nutrient, the study will have power of more than 99% to yield a statistically significant result. This computation assumes the most conservative average MoCA score and its standard deviation of 25.2 (SD = 3.2) found in the previous validation studies and the mean difference of 0.7 over 3 years. It is also assumed that this effect size is reasonable, in the sense that an effect of this magnitude could be anticipated in this field of research. If we allow for two-way interaction between the effects of vitamin D and omega-3 fatty acids, but no interaction with the effect of the exercise program, then individual arms can still be combined and sample sizes of at least 451 and 451 will be available to isolate the pure effects of each intervention and the power remains at 90%.

Immunity: *significant difference in the rate of any infection*

Based on an ongoing study in community-dwelling seniors with a mean age of 73 years (259) we observed a total of 106 infections (any) during 18104 person-days with the same follow-up structure as in DO-HEALTH (clinical visits plus phone calls). Thus, we estimate the rate of any infections to be 2.13 per observed person year. In the pilot study to DO-HEALTH, we observed a significant reduction of severe infections that led to hospital admission in seniors with acute hip fracture; the effect size over 12 month was 90% with 2000 IU vitamin D compared to 800 IU vitamin D (234). We assume a conservative reduction of incidence of any infection by 15% in DO-HEALTH.

For DO-HEALTH, as in the pilot RCT, we assume a Poisson distribution for the number of infections, and we will use a Poisson regression analysis aiming to compare the number of infections between groups at the end of the trial. For each group, we anticipate that there will be 2709 person-years of follow-up. This was calculated for each group assuming that 68% of the 1075 subjects in the arm will have 3 years of follow-up, while 32% of the 1075 subjects who withdraw early will have on average 1.5 years of follow-up.

Poisson assumption: E(x) = Var(x), thus the standard deviation (s) is not equal between the groups.

Assumptions:

$\mu_1 = 2.13$ $\mu_2 = 1.81$
$\mu_1 - \mu_2 = 0.32$ (15%)
n_1 (number of person yrs. of f-u) = 2709 $n_2 = 2709$

s_1 (standard deviation) = $\sqrt{2.13}$ = 1.46 s_2 = $\sqrt{1.81}$ = 1.35

With the criterion for significance set at α = 0.01, a 2-tailed test interpretation and effective sample sizes of 903 and 903 for the main effect of vitamin D, the study will have power of 99% to yield a statistically significant result. This computation assumes that the mean difference is 0.32 (corresponding to means of 2.13 vs. 1.81) and that the standard deviations are 1.46 and 1.35 respectively. This effect was selected as the minimal clinically significant. If there is effect modification between vitamin D and omega-3, then the person years of follow-up become 1355 and 1355 based on the smaller sample sizes, but our power to isolate the pure effect of vitamin D remains very high and over 90%.

Table 3. Power considerations for the primary endpoints.*

Endpoint	Minimum detectable difference†	Standard Deviation	Alpha	Power with no interact., N = 1806 (903 + 903)	Power with interaction, N = 902 (451 + 451)
Bone:‡					
Incidence rate of any non-vertebral fracture including hip fractures (proportion)	0.073¶	–	0.01	0.99	0.80
Muscle:**					
lower extremity function (mean SPPB score)	0.40	1.41	0.01	0.99	0.97
Cardiovascular:**					
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
Brain:**					
Cognitive function (mean MMSE score)	0.7	2.3	0.01	0.99	0.90
Immunity:††					
Incidence rate of any infection	0.32	s_1 = 1.46 s_2 = 1.35	0.01	0.99	0.90

* All assumptions about means and standard deviations of the parameters are based on the results of pilot trials to DO-HEALTH (7, 8, 23) and research of other authors (58, 59, 84).
† Minimum detectable difference was assumed as a minimum difference that is clinically significant.
‡ Power calculations are based on the Z-test for difference between two proportions.
¶ Estimated baseline prevalence of any non-vertebral fractures incidence rate / IR = 0.14.
** Power calculations are based on the two-sample t-test assuming equal variances.
†† Power calculations are based on Poisson regression assuming non-equal variances.

11.2.2. Power considerations for secondary endpoints

The DO-HEALTH trial will have at least 80% power to detect minimal clinically significant differences for secondary endpoints regarding the interventions. The most critical secondary endpoint is the risk of hip fractures. The expected rate of hip fractures in DO-HEALTH is 6% (IR = 0.06) and is based on the placebo groups of all double-blind trials that enrolled individuals age 70+ from our meta-analysis of individual participant data pooled from 11 double-blind RCTs of vitamin D and fracture prevention (8), while the effect size is based on the pilot trial for DO-HEALTH where we documented a non-significant 48% reduction of hip fracture risk comparing 2000 IU vitamin D to 800 IU vitamin D in 173 seniors over a 12 month follow-up (7). With the criterion for significance set at α = 0.05, and a 2-tailed test interpretation, effective sample sizes of

903 and 903 for the main effect of vitamin D, we will have power of 84% to detect a minimally clinically significant difference in proportions (0.029).

11.2.3. **Power considerations for exploratory endpoints**

We propose several exploratory endpoints to support the primary endpoints of DO-HEALTH. DO-HEALTH may not be sufficiently large to be powered for the exploratory endpoints listed, however, these endpoints are still important to assess as they will serve as pilot data for future studies and will allow the comparison of DO-HEALTH findings with the VITAL study findings. For the total cancer endpoint, DO-HEALTH may have sufficient power based on a recent double-blind randomized trial, which showed a significant reduction of total cancer risk in 1179 community-dwelling healthy postmenopausal women with vitamin D (126). Using the rates of cancer and the effect size from this trial, DO-HEALTH would have 95% power to yield a statistically significant result for the main effect of vitamin D and with a 32% dropout.

Fracture healing is a novel endpoint and DO-HEALTH will be one of the first RCTs to assess fracture healing at 3 levels in individuals with incident major osteoporotic fractures at the arm (shoulder, humerus, forearm) and leg (hip, femur, ankle): (a) *primary fracture healing endpoint*: clinical fracture healing with 3 additional phone calls at 6, 12, 18 weeks after the fracture assessing the PROMIS-HAQ (b) *secondary fracture healing endpoint*: observed functional fracture healing measured with the Short Physical Performance Test Battery and grip strength at regular 12, 24, 36 month visits (c) *exploratory fracture healing endpoint*: radiological fracture healing with independent assessment of early (6-8 weeks) and late (12 to 14 weeks) consolidation assessment based on standard x-rays. The fracture healing study is an ancillary study with pending funding by the AO foundation and the endpoint is co-led by the DO-HEALTH partner at the University of Basel (N. Suhm) and the Coordinating DO-HEALTH Centre (H.A. Bischoff-Ferrari) supported by all DO-HEALTH recruitment partners. Extra staff time / logistics / programming / analyses will be funded by the ancillary funds through the AO foundation.

11.3. Randomization

11.3.1. **General information**

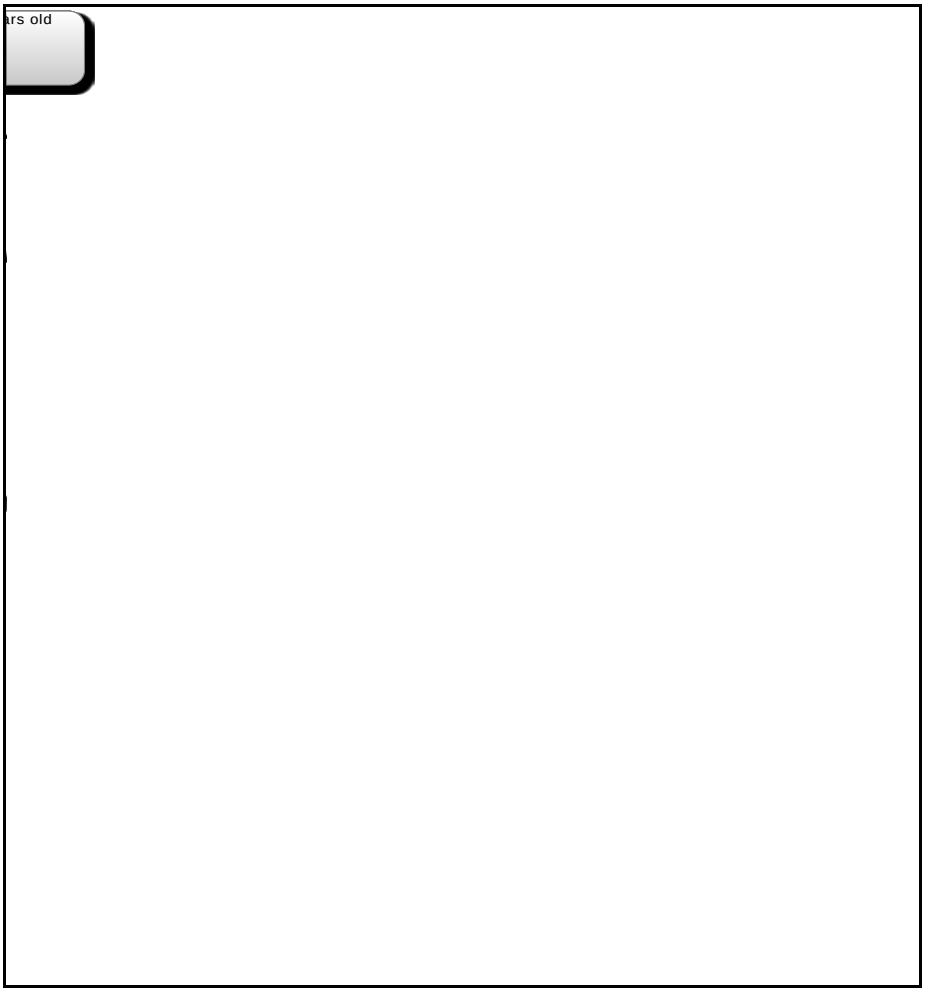
Participants are randomized into the trial during the baseline visit. Randomization will be computer-based (DO-HEALTH randomization software). Stratified block randomization strategy will be used (block size - 16 individuals). Willing and eligible participants will be randomized to one of the eight treatment groups stratified by the study centre, low trauma fall (definition see chapter 7.2 on page 40) during previous 12 months prior to the randomization day (yes/no), age (70 – 84 and 85+), and gender. Within each group, treatment assignments will be generated in blocks of sixteen individuals, with two individuals in each of the eight treatment combinations. Each study centre will be forced to keep a minimum balance between participants without falls and participants with at least one fall in the last year prior to enrollment, with at least 40% of fallers. The centers will also be required to monitor balance in the age and gender strata. If gross imbalance (less than 30% in one of the strata) is detected in age and gender strata, measures will be taken to boost recruitment of participants in underrepresented strata. The DO-HEALTH randomization software will provide a simple summary statistic for each recruitment centre by stratification variable to monitor recruitment progress for each centre and indicate what subgroup needs to be target in the future enrollment.

11.3.2. Prerequisites for randomization

Before randomization can be conducted, the following procedures must have been completed:

1. Participant's Informed consent form is signed
2. Serum calcium was measured and the measurement was below 2.6 mmol/L
3. Serum creatinine was measured, creatinine clearance was calculated according to the Cockcroft-Gault formula (286), and was above 15 ml/min.
4. The study MD has seen the participant and confirmed all inclusion and exclusion criteria;
5. The mini mental state examination (MMSE) was applied with the participant and the participant reached a score > 24
6. The participant is able to walk 10 meters with or without walking aid and is able to get in and out of a chair without help (simple screening test by the study nurse).

Figure 8. Determining randomization strata.



11.3.3. Randomization procedure

Randomization will be computer-based (DO-HEALTH randomization software). Each centre will have a log-in and will be asked to provide the stratification variables of the new participant. Based on this information, the software will issue the subject ID and intervention/ exercise package code. In case of an internet access problem, the recruitment site may call the coordinating centre at the University of Zurich to perform the

randomization. For each randomization centre, a separate but identical structure will be applied to identify the strata for randomization. See Figure 8 as an example: randomization strata for a 72 year-old woman who fell during last year is highlighted. The recruitment centre submits information on prior fall, gender, and age to identify the strata, and will receive the subject ID and intervention/exercise package code from the DO-HEALTH randomization software. Each of the recruitment centers has a pre-coded supply of study intervention (12 bottles per code per year) and a supply of exercise material (video + written manual + rubber bands). The participant is considered randomized once the subject ID has been issued and assigned to him/her. The intervention package code is used to match the study intervention to the subject ID after randomization. Each bottle of the study intervention will be labeled with the ID in addition to the intervention code. At each visit participant will receive 12 bottles of the study intervention to provide the year's supply.

11.4. Interim Analyses

No interim analyses are foreseen. Interim analyses may be performed only if the Data Safety and Monitoring Board (DSMB) deems them necessary and requests them for safety reasons. In this case, the interim analyses will be performed only to the extent that satisfies the request of the DSMB.

An independent **Data Safety and Monitoring Board** (DSMB) will be assembled consisting of 3 experts: a biostatistician/epidemiologist, a geriatrician/physician, a scientist with a background in nutrition/physical activity. The DSMB will be independent without any other relationship to the DO-HEALTH study, no financial interest in the outcome of the study, and will be excluded from authorship of study findings. The DSMB will examine the progress and safety data of the DO-HEALTH clinical trial to recommend continuation, alteration of study design or early termination, as appropriate. To do so, the DSMB may review unblinded study information (on patient level or treatment group level). The DSMB will be instructed on interim (baseline associations) findings (Head biostatistician, PI and data management team) and will provide recommendations to the Coordinator and Governing Board of DO-HEALTH. The DO-HEALTH DSMB will be established by December 2013 supported by a standard operating procedure manual , meet in yearly intervals starting in February 2014, when about 50% of the DO-HEALTH participants will be recruited. Note that the trial was designed within a network of geriatricians and scientists experienced in the clinical care of seniors and safety procedures related to the interventions tested in DO-HEALTH.

12. (Serious) Adverse Events

12.1. Definition of (Serious) Adverse Events

12.1.1. **Adverse events**

Adverse events (AEs) are defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal study product, whether or not related to the medicinal study product. An AE may also consist of a new disease, an exacerbation of a pre-existing illness or condition, a recurrence of an intermittent illness or condition, a set of related signs or symptoms, or a single sign or symptom.

AEs observed by the investigator and/or reported by the subject must be reported in the CRF during the entire study period, i.e. the period of time from the first (= signature of informed consent) to the last protocol-specific procedure regardless of the medicinal study product relation assessment.

For all AEs, sufficient information will be pursued and/or obtained so as to permit an adequate determination of the outcome of the event (i.e., whether the event should be classified as an SAE) and an assessment of the casual relationship between the AE and the investigational intervention or study treatment(s).

Whenever available, the underlying disease or condition for which a therapeutic or diagnostic procedure is required should be reported as the AE term. Surgeries or other invasive procedures that had already been planned prior to the start of the study do not have to be documented as AEs. These planned procedures will be recorded in the CRF by the investigator at the baseline visit. It is not important if the condition was known before enrolment, only if the procedure was planned before.

12.1.2. **Serious adverse event**

An SAE is any untoward medical occurrence that at any dose

- results in death,
- is life-threatening,
- requires subject hospitalization or prolongation of current hospitalization,
- results 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.

Any other medically important condition that may be not immediately life-threatening or results in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the outcomes listed above should also usually (i.e. based on medical and scientific judgment) be considered serious. For

example: intensive treatment at home for allergic bronchospasm; certain laboratory abnormalities (e.g. blood dyscrasias); convulsions that do not result in hospitalisation; development of dependency or abuse.

12.2. Recording of (Serious) Adverse Events

Clinical study subjects will be routinely questioned about AEs at study visits. The well-being of the subjects will be ascertained by neutral questioning ("How are you?"). The investigator is responsible for reporting all AEs occurring during the course of the study.

All observed or volunteered adverse events (serious or non-serious) and abnormal test findings, regardless of treatment group or suspected causal relationship to the investigational intervention or study treatment(s) will be recorded in the CRF.

AEs or abnormal test findings felt to be associated with the study treatment(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

An abnormal test finding will be classified as an AE if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention, including significant additional concomitant treatment or other therapy
  Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE
- The test finding leads to a change in study dosing or discontinuation of subject participation in the clinical study.

All AEs, serious and non-serious, will be fully documented on the appropriate CRF. For each AE, the investigator will provide the onset, duration, intensity, treatment required, outcome and action taken with the investigational product.

The intensity of AEs will be 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).

The investigator will determine the relationship of the investigational intervention to all AEs as defined on the Adverse Event Reporting Form.

12.3. Assessment of (Serious) Adverse Events

The investigator at the respective DO-HEALTH recruitment site will promptly review documented AEs and abnormal test findings to determine if:

- the abnormal test finding should be classified as an AE
- if there is a reasonable possibility that the AE was caused by the investigational intervention or study treatment(s), and
- if the AE meets the criteria for an SAE.

The assessment by the investigator with regard to the study intervention relation is done according to the following definitions:

- Unlikely relation: An AE, whose
  - temporal relationship to trial intervention makes a causal relationship improbable and
  - in which other interventions or chemicals or underlying disease provide plausible explanations.

- Possible relation: An AE, which
  - occurs within a reasonable time sequence to administration of the trial intervention but
  - could also be explained by concurrent disease or other interventions or chemicals.

  Information on withdrawal from intervention may be lacking or unclear.

- Likely relation: An AE, which
  - occurs within a reasonable time sequence to administration of the intervention,
  - is unlikely to be attributed to concurrent disease or other interventions or chemicals, and
  - follows a clinically reasonable response on withdrawal (dechallenge).

  Rechallenge information is not required to fulfill this definition.

- Certain relation: An AE, which
  - occurs in a plausible time relationship to intervention administration and
  - cannot be explained by concurrent disease or other interventions or chemicals.
  - the response to withdrawal of the intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.

Adverse event classification will be done according to the Investigator's Brochure of the investigated dietary supplements. If the study MD at the respective recruitment site is uncertain how to classify the event, the Monitor (Pharmalys) or the central study MD at the DO-HEALTH coordinating centre (University of Zurich) can be contacted for support.

12.4. Reporting of Serious Adverse Events

The procedure described in the following chapters applies in general to all DO-HEALTH centers, but adaptations to local requirements are possible if that is required by local legislation. Detailed procedures will be available to the centers (SOPs).

The Principle Investigator at the respective recruitment site is responsible for SAE reporting to the Sponsor and – depending on local legislation – to the responsible regulatory authority and/or to the IEC, respectively, according to the following details.

The Recruitment Center Principal Investigator (here following also defined as "Local PI") **at each recruitment site is responsible for:**

- Reporting to the Sponsor any SAE within 24 hours
- Reporting to IEC immediately, i.e. within 24 hours, any SAE that resulted in death
- Compliance with the regulatory requirements of the responsible regulatory authority regarding prompt reporting of unexpected SAEs for which a causal relationship with the study intervention or device cannot be ruled out.

- Reporting to the IEC, the responsible regulatory authority and to the Sponsor (Prof. Heike Bischoff-Ferrari) of fatal and life-threatening SAEs if evaluated as "suspected", "unexpected" and "intervention related" (SUSARs):
  - without delay and no later than 7 calendar days following awareness that event meets criteria for a SUSAR;
  - follow-up information regarding the fatal SAE within further 8 calendar days.
- Reporting to the IEC, the regulatory responsible authority and to the Sponsor (Prof. Heike Bischoff-Ferrari) of non-fatal and not life-threatening SAEs if evaluated as "suspected", "unexpected" and "intervention related" (SUSARs):
  - promptly and no later than 15 calendar days following awareness that event meets criteria for a SUSAR.

An unexpected SAE refers to any AE, the nature or severity of which is not consistent with the applicable product information (Investigator's brochure).

- Sending yearly safety reports, starting one year after the date of notification to the responsible local authority and to the Sponsor (Prof. Heike Bischoff-Ferrari). These reports should contain:
  - A concise critical summary of the safety profile of the intervention studied as well as the safety issues that have arisen;
  - A listing of all adverse events related to study intervention and SUSARs that have occurred in the respective country

The Sponsor and Principal Investigator (University of Zurich, Prof. H. Bischoff-Ferrari) is responsible for:

- Making the SUSAR information provided by each local sponsor representative available to all other sites
- Distributing a "global" yearly safety report to local Sponsor representatives, this will include information on adverse events related to study intervention and SUSARs collected across all centres, starting one year from the ethics committee approval date. The report can be submitted to local authorities, as required.

12.5. Follow-up of (Serious) Adverse Events

Subjects terminating the study (either regularly or prematurely) with

- reported ongoing SAE, or
- any ongoing AEs of laboratory values or of vital signs being beyond the alert limit

will return for a follow-up investigation. This visit will take place up to 30 days after terminating the treatment period. Follow-up information on the outcome will be recorded on the respective AE page in the CRF. All other information has to be documented in the source documents. Source data has to be available upon request.

For any AEs the outcome "unknown" is not acceptable, except if attempts to collect the information have been made and documented. In case of subjects lost to follow-up, efforts should be made and documented

to contact the subject to encourage him/her to continue study participation as scheduled. In case of minor AEs a telephone call to the subject may be acceptable.

All new SAE that the investigators will be notified of within 30 days after discontinuation of study intervention have to be reported in appropriate report forms and in the CRF if required.

Follow-up investigations may also be necessary according to the investigator's medical judgment even if the subject has no AE at the end of the study. However, information related to these investigations does not have to be documented in the CRF but must be noted in the source documentation.

12.6. Expected Frequency and Intensity

Based on our experience with the DO-HEALTH pilot studies (7-9, 23) and prior research by other investigators that administered vitamin D and omega-3 fatty acids [12, 13] dietary supplements in doses and regimens comparable to those used in this study, we expect that adverse effects will be rare and their intensity will be mild and will not go beyond minimal symptoms, slightly, if at all, impairing general wellbeing. The results of the DO-HEALTH pilot trial show that the home exercise program is well tolerated even by frail seniors and poses low risk (7). Potential adverse effects associated with the program are also expected to be rare and of mild intensity.

We will also **compare the incidence of potential side effects** in the active *vs.* control groups for each treatment, including the incidence hypercalcemia and kidney stones with vitamin D assignment (Women's Health Initiative calcium-vitamin D findings (22)) and the incidence of GI symptoms, skin abnormalities, and bleeding with omega-3 fats assignment (JELIS findings (15)).

13. Ethical Considerations

13.1. Ethical Requirements and Protection of Human Participants

Conduct of DO-HEALTH is founded on the protection of human rights and the dignity of the human beings (Declaration of Helsinki, 2000, World Medical Association (287)). Therefore, in the DO-HEALTH clinical trial considerations related to the well-being of human subjects will take precedence over the interests of science and society according to the Clause 5 of the Declaration.

Protection of the trial subjects is safeguarded through risk assessment based on the results of pre-clinical and validation experiments prior to any clinical trial and consideration by ethics committees and Member States' competent authorities. Rules on the protection of personal data (Directive 2001/20/EC) will be also carefully applied. In addition, ICH Good Clinical Practice (GCP) is a set of internationally recognized ethical and scientific quality requirements, which will be observed for designing and conducting the DO-HEALTH trial, recording the data, and reporting the trial results.

Compliance with this good practice guidance provides assurance that the rights, safety and well-being of the trial subjects are protected, and that the results of the trial are credible.

Standards for clinical trials are set out in:

- For non-EU study centres: World Medical Association, Declaration of Helsinki 2000 (287)
- For EU study centres: World Medical Association, Declaration of Helsinki 1996
- World Health Organization, Guidelines for Good Clinical Practice for Trials on Pharmaceutical Products (1995)
- International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines for Good Clinical Practice (1996)
- "Oviedo Convention" - Council of Europe Convention on Human Rights and Biomedicine and it's additional protocols, particularly the Additional Protocol concerning Biomedical Research (January 2005)
- CIOMS (Council for International Organizations of Medical Sciences) International Ethical Guidelines for Biomedical Research Involving Human Subjects (2002)
- Universal Declaration of Human Rights (1948)
- UN Convention on the Rights of the Child (1989)
- EU Clinical Trials Directive (2001/20/EC)

DO-HEALTH will carefully observe all these standards ensuring the highest ethical standards of the trial and protection of health and well-being of its participants.

13.1.1. **Confidentiality**

During the DO-HEALTH clinical program, all information collected as part of the study will be treated confidentially. All study data related to the participants will be coded when registered, and all data will be entered into the DO-HEALTH data base by subject ID only. Data bank access will be strictly regulated and

centralized (DO-HEALTH data management team) and all analyses will be performed centrally at the DO-HEALTH coordinating centre at the University of Zurich.

The subject will be made aware of (and give consent to) the fact that monitors, auditors, Ethics Committee, and regulatory authorities will be granted direct access to the subjects medical records without violating subject confidentiality and to the extent permitted by applicable regulations. The subject will be informed that by signing the informed consent form, the subject authorizes such access. All subjects also consent to a medical release form on all health-related treatment procedures during the follow-up of the DO-HEALTH project. All subjects are asked to give an explicit consent that in case of their drop-out, all collected material including blood samples can be analyzed for the endpoints in the DO-HEALTH trial and that their biological samples can be stored for future analyses in the DO-HEALTH biobank.

All information generated in this study will be considered highly confidential and will not be disclosed to any persons not directly concerned with the study without written prior permission from the Sponsor. However, authorized regulatory officials and sponsor personnel (or their representatives) will be allowed full access to inspect and copy the records. All study interventions, subject's body fluids, and/or other materials collected shall be used solely in accordance with this protocol, unless otherwise agreed to in writing by the Sponsor (PI of DO-HEALTH).

Subjects will be identified only by unique subject ID numbers in the CRFs. Their full names may, however, be made known to a regulatory agency or other authorized officials if necessary.

To ensure the highest standard of data safety, DO-HEALTH dedicates a work package to data management and safety. Data storage, management, and handling will be protected in accordance with European Commission and national directives that are listed in Section 13.2. Data will be collected electronically and sent to the data management centre immediately. Daily backups of the database allow recovery of the most recent data in case the data are inadvertently lost. These backups are stored in a fireproof armored filling cabinet. All databases are password protected.

Furthermore, subjects' privacy and protection of personal data will be safeguarded according to Directive 95/46/EEC dealing with:

- Coding, storage and protection of identity, biological material, recorded data: the storage of personalized identifiers besides a pseudo-anonymized coding of results
- Right of the subject to get information on what data are recorded, how they are recorded, who will be responsible for keeping the data and who will have access to the data

In publications with reference to this study, the subject's identity will remain confidential.

All data will be processed and analyzed centrally at the University of Zurich in Switzerland (DO-HEALTH coordinating Centre) where privacy legislation will be strictly maintained.

Regarding the transfer or disclosure of results to third parties, only coded data are authorized to be communicated. That is to say that, all personal identifiers will be removed of any data that come from the recruitment centers to the central database before transfer. For the confirmation procedure of cases by the endpoints Committee of physicians, blinded to the randomized treatment assignment, or the external DXA

assessment (vertebral morphometry) or X-ray assessment in DO-HEALTH (ancillary fracture healing study) the identifiers on the DXA or X-ray reports will be replaced by unique identifiers that are separately and securely held by data management team at the DO-HEALTH coordinating centre at the University of Zurich. Notably, any reports sent to the coordinating centre will be based on subject ID only (including X-rays and DXA reports).

13.1.2. **Informed consent**

Each participant will give his/ her written informed consent before participating in the clinical trial or any trial-related procedure(s). The signed informed consents will be retained by the investigator at the recruitment site and made available (for review only) to the study monitor, auditor, and inspector. All information related to the clinical trial (trial objectives, possible risks, harms and inconveniences related to conduct of the trial as well as all the conditions under which it will be conducted, the purpose of collection and storage of data/biological material, methods and techniques used and measures taken to protect confidentiality, procedures related to data access and storage, duration of data storage, and personnel authorized to access the data) will be provided to the participants before their decision to participate in the trial or abstain from participation. See section 7.10 on written informed consent. The participant information and written informed consent will be translated in all 3 DO-HEALTH languages and are attached to this protocol in the English version. To ensure that the participants fully understand the nature and purpose of the study, the participant information has been pre-tested in a subgroup of the target population.

13.1.3. **Protection of clinical trial participants**

- The DO-HEALTH clinical trial will be conducted in compliances with Directive 2001/20/EC without prejudice to the national provisions on the protection of clinical trial participants if they are more comprehensive than the provisions of this Directive.
- The clinical trial can be undertaken since the foreseeable risks and inconveniences have already been weighed against the anticipated benefit for the individual trial participant and other present and future participants. The clinical trial will be initiated only if the Ethics Committee and/or the competent authority come to the conclusion that the anticipated therapeutic and public health benefits justify the risks.
- The investigator is responsible for ensuring that the subjects fully understand the nature and purpose of the study. The trial participants will provide written informed consent in person and will understand the objectives, possible risks, harms and inconveniences related to conduct of the trial as well as all the conditions under which it will be conducted, the purpose of collection and storage of data/biological material, methods and techniques used, measures taken to protect confidentiality, staff members who will access the data, and duration of the data storage period. The participant will be also informed of his/ her right to withdraw from the trial at any time. It will be made clear that refusal to participate or withdrawal from the study at any stage of the trial will not induce any prejudice to the participant's subsequent care. Lastly, participants will be allowed sufficient time to decide whether or not they wish to participate in the trial. The rights of the participant for physical and mental integrity, for privacy and protection of the data concerning him/ her will be safeguarded in accordance with Directive 95/46/EC.
- Provision has been made for insurance or indemnity to cover the liability of the investigator at the recruitment site. In accordance with ICH GCP Guidelines and applicable regulatory requirements,

the sponsors will ensure that adequate participant insurance is in place at each clinical site for the period of the study, which will be confirmed by the monitor. Due to the nature of the clinical trial (Phase III), participants will not receive any financial reward or compensation. More than 30% of the elderly participants have cognitive impairment. This means a relevant subgroup amongst eligible participants cannot be included in high quality studies when formal informed consent is required. Thus, we accept a selection bias by excluding such participants with cognitive impairment by means of the study protocol. We require that a prospective participant have a Mini Mental State Examination Score of at least 24 to be eligible for participation in the trial.

13.1.4. **Risk – benefit assessment**

Potential Risks

The **physical risks** in the trial are associated with: 1) the study treatments (vitamin D, omega-3 fatty acids, and physical activity program); 2) blood draws; 3) DXA scan.

Vitamin D: We chose 2000 IU Vitamin D per day as the supplement dose for vitamin D. This dose proved to be safe in the DO-HEALTH pilot trial (7), even if combined with a calcium supplement of 1000 mg per day (which will not be provided in DO-HEALTH). The safety of 2000 IU vitamin D per day is further supported by our benefit-risk analysis where a safe upper intake level of 10,000 IU vitamin D per day was estimated (1); in November 2010, the Institute of Medicine (IOM) increased the safe upper intake limit for vitamin D from 2000 to 4000 IU per day (51). Because we will exclude from the trial persons who report supplemental vitamin D intakes >800 IU/d, the selected dose ensures that no participants will be taking a supplemental vitamin D dose above 2800 IU, which is below the 4000 IU defined as the safe upper intake level by the IOM. To further ensure participants' safety, we will exclude from the trial persons with hypercalcemia (serum calcium > 2.6 mmol/l), renal failure, sarcoidosis or other granulomatous disease, and will obtain serum calcium levels as safety endpoints in all clinical visits (baseline, 12, 24, 36 months). Based on recent data regarding the potentially increased cardiovascular risk with high dose calcium supplementation (21) and as an additional safety insurance for anti-fracture efficacy with high-dose vitamin D (8), we limit the personal calcium supplement intake allowance to 500 mg per day during the trial in all participants and recommend sufficient calcium intake from dietary sources.

Potential side effects of the vitamin D will be assessed on each in person follow-up (3-monthly interval). These side effects include physician diagnosis of hypercalcemia. We will also monitor as a safety endpoint the self-reported incidence of kidney stones although vitamin D alone without calcium supplementation has not been associated with kidney stones (see our benefit-risk assessment (9) performed in preparation of DO-HEALTH).

Omega-3 fatty acids: 1 g of omega-3 fatty acids (EPA to DHA ratio 1:2) per day was chosen as a supplement dose for this trial. This dose is recommended by the American Heart Association for cardio-protection and was beneficial in one large secondary prevention trial with minimal side effects (199). Omega-3 fatty acids in total doses ranging from 850 mg/d to 1.8 g/d were used as treatments in several clinical trials (15, 199, 288) and no serious adverse effects related to the omega-3 fatty acids treatment were reported. For omega-3 fats, there are no specific exclusions necessary except for those taking the supplement in the 3 months prior to recruitment or unwilling to refrain from their use during the trial. Health risks associated with omega-3 fats are believed to be minimal. The FDA has concluded that marine omega-3 fatty acid doses of up to 3 g/d

are “Generally Recognized as Safe” (289). Although omega-3 fatty acids have potential antithrombotic effects, systematic reviews of data from small, short-term trials suggest that omega-3 fatty acid supplements do not increase the risk of clinically significant bleeding at doses of up to 4 g/d, even in combination with anticoagulant interventions such as aspirin or warfarin (18, 19). The JELIS investigators did report an increase in bleeding events with 1.8g EPA (1.1%) as compared with placebo (0.6%; p=0.0006), but this dose is higher than what we propose (15). Other concerns include an unpleasant aftertaste (notably this is less of a concern with the algae versus fish-based omega-3 fats), gastrointestinal (GI) disturbance (e.g., nausea or diarrhea), and fish fat oxidation (i.e., rancidity), all of which contribute to intolerance (19, 102). In order to overcome the unpleasant aftertaste, the capsule will be coated.

Potential side effects of omega-3 fats will be assessed on each in person follow-up (3-monthly interval). These side effects include gastrointestinal (GI) upset (presence or absence of symptoms of peptic ulcer, nausea, constipation, diarrhea), bleeding (any GI bleed, GI bleeding requiring transfusion, hematuria, easy bruising, epistaxis), skin eruptions, and physician diagnosis of atrial fibrillation or other irregular rhythms. The potential “unpleasant aftertaste”, which is much reduced by using algae-based instead of fish-oil based omega-3 fatty acids, is further reduced by the capsule coating in DO-HEALTH, but would be covered in the assessment of comparative effectiveness where we ask why and why not participants adhere to the study intervention.

Physical activity program: The simple exercise program that will be used for this trial was tested in the DO-HEALTH pilot trial (7) and proved to be feasible and well tolerated even among frail seniors. Potential side effects will be assessed on each in person contact (3-monthly interval). These include increased muscle pain or increased joint pain and theoretical circulatory disturbance, dizziness, falls and tendon lesions.

Blood draw: The blood draw procedure requires that a needle be inserted into an arm and blood withdrawn. Almost all donors experience slight pain at the site of the needle insertion and some may develop a small bruise. Universal precautions will be followed for procuring and handling all biological samples.

DXA scans (performed only in a subset of participants from 4 centers): At each visit at the recruitment centre, it is planned to do a bone density test, a vertebral fracture assessment (vertebral morphometry) and body composition DXA scan which will involve irradiation. The **total** amount of radiation will be **72 microSievert** (µSv) for all visits (3 µSv for hip BMD, 3 µSv for lumbar spine BMD, 5 µSv for whole body BMD, 7 µSv for vertebral morphometry: total 18 µSv per visit, i.e. 72 µSv for all 4 visits) . These radiation doses are considered safe for adults. In order to better understand what these amounts mean, here are some examples: natural background radiation – we are all exposed to – is ~2400 µSv per year. During a transatlantic flight, the exposure is ~5 µSv per flight hour, during a chest X-ray is ~100 µSv, and during a chest CT-scan is ~8000 µSv.

For each clinical center, ethical approval from local and/or national authorities is required for conduct of the study. In order to obtain approval from the Ethical Committees, investigators will provide a valid authorization request supported by the monitor and the coordinating DO-HEALTH centre. The protocol to be submitted for ethical approval at all study sites has been prepared by the DO-HEALTH PI (Prof. Heike Bischoff-Ferrari) and coordinating centre staff at the Centre on Aging and Mobility at the University of Zurich. Its contents were reviewed and confirmed by all partners of DO-HEALTH.

The study will only start at any of the recruitment centers after written approval has been received from an Institutional Review Board / Ethics Committee (IRB/EC), which operates according to ICH GCP Guidelines. The written approval and the names and qualifications of members of the IRB/EC must be made available to the sponsor (Prof. Heike A. Bischoff-Ferrari) before the study can start. The study site Principal Investigator is, together with the sponsor, responsible for submission to and communication with the IRB/EC.

In addition, the study site Principal Investigator should conduct the study in accordance with the protocol, the Declaration of Helsinki (version 1996) and the ICH GCP Guidelines (CPMP/ICH/135/95). The study site study site Principal Investigator and the sponsor and DO-HEALTH Principal Investigator (Prof. Heike Bischoff-Ferrari) will sign the protocol and Clinical Trial Agreement to confirm this. All recruitment centers will perform all tasks described in this protocol.

German clinical centers will also need approval of German Radiation Protection Office (BfS) for all radiological measurements within DO-HEALTH (IDXA).

13.2. Legal Requirements

13.2.1. **National legislation**

The 23 partners will conform to current legislation and regulations in the countries where the research will be carried out. Where required by national legislation or rules, they must seek the approval of the relevant ethics committees prior to start of the RTD activities that raise ethical issues.

13.2.2. **EU legislation**

DO-HEALTH partners will conform to relevant EU legislation such as:

- The Charter of Fundamental Rights of the EU
- Directive 95/46/EC of October 24, 1995 on the protection of individuals with regards to processing of personal data and the movement of such data
- Directive 2001/20/EC of April 4, 2001 on clinical good practice
- Directive 2001/20/EC of the European Parliament and of the Council of April 4, 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use
- Detailed guidance on the application format and documentation to be submitted in an application for an Ethics Committee opinion
- Detailed guidance for the request for authorization of a clinical trial to the competent authorities
- Directive 2001/83/EC of the European Parliament and of the Council of November 6, 2001 on the Community code relating to medicinal products for human use
- Directive 2003/63/EC of June 25, 2003 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use
- Regulation (EC) No. 1084/2003 of June 3, 2003 concerning the examination of variations to the terms of a marketing authorization for medicinal products for human use and veterinary medicinal products granted by a competent authority of a Member State

- Regulation (EC) No. 1085/2003 of June 3, 2003 concerning the examination of variations to the terms of a marketing authorization for medicinal products for human use and veterinary medicinal products falling within the scope of Council Regulation (EEC) No. 2309/93
- Directive 98/44/EC of the European Parliament and of the Council of July 6, 1998 on the legal protection of biotechnological inventions
- Directive 86/609/EEC of November 24, 1986 on the protection of animals
- Directive 86/609/EEC of November 24, 1986 on the protection of animals used for experimental and other scientific purposes
- Protocol on Protection and welfare of animals (protocol to the Amsterdam Treaty)
- Directive 90/219/EEC of April 23, 1990 on the contained use of genetically modified microorganisms
- Directive 2005/28/EC on Good Clinical Practice (GCP)
- Directive 98/81/EC of October 26, 1998 amending Directive 90/219/EEC on the contained use of genetically modified microorganisms
- Directive 2001/18/EC of the European Parliament and of the Council of March 12, 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC
- Regulation (EC) No 1946/2003 of the European Parliament and of the Council of July 15, 2003 on transboundary movements of genetically modified organisms (Text with EEA relevance)
- Regulation (EC) No 65/2004 of January 14, 2004 establishing a system for the development and assignment of unique identifiers for genetically modified organisms
- Directive 2000/54/EC of the European Parliament and of the Council of September 18, 2000 on the protection of workers from the risks related to exposure to biological agents at work (7th individual directive within the meaning of Article 16(1) of Directive 89/391/EC)
- Directive 2004/23/EC of the European Parliament and of the Council on Setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells", code number 2002/0128 (COD), Strasbourg, March 31, 2004

13.2.3. **International legislation**

DO-HEALTH partners will respect the following international conventions and declarations:

- For non-EU study centres: Declaration of Helsinki in its latest version
- For EU study centres: Declaration of Helsinki as amended on 1996
- Convention of the Council of Europe on Human Rights and Biomedicine signed in Oviedo on 4 April 1997, and the Additional Protocol on the Prohibition of Cloning Human Beings signed in Paris on January 12, 1998
- UN Convention on the Rights of the Child
- Universal Declaration on the human genome and human rights adopted by UNESCO

14. Monitoring

14.1. Routine Monitoring

As this is a multi-centre clinical trial (7 recruitment sites in 5 countries), DO-HEALTH will include a monitoring partner (Pharmalys) with significant experience in monitoring international trials. With Pharmalys being a partner to DO-HEALTH, GCP guideline compliance will be a high priority in DO-HEALTH, with implementation and monitoring at the initiation and each follow-up clinical visit – and at each of the 7 recruitment sites. The reports of the monitor will be reviewed by the Data Safety and Monitoring Board and the Government Board. Monitoring is a priority in DO-HEALTH to ensure the DO-HEALTH trial quality is outstanding and consistent across all recruitment sites.

After the initiation visit, regular site monitoring visits will occur for all 4 clinical visits followed by a close-out visit at the end of the trial – at each of the 7 recruitment sites. The monitor will support the recruitment centers in the application for authorization of DO-HEALTH by the national competent authorities and its approval by the ethical committee of each participating country. The monitor will review at each site visit, the ongoing collection and maintenance of study master files. The monitor will review the ongoing shipment of monitored and collected CRF pages to the Coordinating Centre at the University of Zurich for data management. At the end of the trial, the monitor will make a close-out visit at all 7 study centers to ensure that all documentation is complete.

14.2. Audit and Inspections

In addition to the monitor, regulatory authorities worldwide may also inspect the investigator during or after the study period. The investigator should contact the sponsor immediately if this occurs, and must fully cooperate with the inspections conducted at a reasonable time in a reasonable manner.

15. Endpoint ascertainment and validation of self-reported endpoints in DO-HEALTH

For participants who self-report a SAE and/or a fracture that classifies for the ancillary fracture healing study a copy of the written informed consent form will be sent as a medical release form authorizing DO-HEALTH staff to obtain hospital/physician records. The ascertainment of the medical record must be performed by the respective recruitment centre after documentation of the self-reported event. Validation of all kind of events will be conducted during the last 3 months of follow-up. If there is no response by the treating hospital/physician within 1 month, a second request will be mailed, followed by a phone call (each step will be documented in the DO-HEALTH electronic data management system).

After medical records are obtained by the respective recruitment centre, (1) the study MD at the respective centre reviews the medical report for completeness and (2) then de-personalizes a copy of the report (only subject ID will be on the report) to be sent to the Coordinating Centre at the University of Zurich. (3) At the University of Zurich, an Endpoints Committee of physicians, blinded to the randomized treatment assignment, will review the report and, using a defined protocol for each endpoint of DO-HEALTH, will confirm or disconfirm the case. Only then the case will be entered in the data – base. For sensitivity analyses, will also record disconfirmed or cases with incomplete confirmation.

For example: A fracture care will be confirmed with an X-ray showing the fracture, an x-ray report describing the fracture, or a medical report describing the fixation of a fracture. The location and type of fracture will be recorded according to a predefined fracture ascertainment system consistent with the VITAL study (provided by an advisor to DO-HEALTH: Prof. Douglas Bauer, UCSF, USA). A cancer diagnosis will be confirmed with histologic or cytologic evidence. In the absence of these diagnostic tests, strong clinical evidence accompanied by radiologic evidence or laboratory markers (e.g., PSA levels) will be used to confirm cancer occurrence. Diagnostic cards will be consistent with the VITAL study. The histologic type, grade, and stage of cancer will be recorded (290). MI will be confirmed using Joint European Society of Cardiology/American College of Cardiology Foundation/ American Heart Association/World Heart Federation (ESC/ACCF/AHA/WHF) Task Force for the Redefinition of Myocardial Infarction criteria.(291) Stroke will be confirmed and categorized according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria (292). Death due to a cardiovascular cause will be confirmed by convincing evidence of a CVD event from all available sources, including death certificates, hospital records, autopsy, and observer accounts (for deaths outside the hospital).

For reported deaths, the validation process will be similar. A condolence letter will be mailed to the family, requesting permission to obtain medical records and a copy of the death certificate. If the family does not provide the death certificate, a copy will be obtained from the state vital records bureau where the participant died. The Independent Physicians Endpoints Committee will review all records relevant to the death and assign an ICD code.

16. Study Termination Procedures

16.1. Premature Study Termination

Twice a year the Data Safety and Monitoring Board (DSMB) will examine the progress of the trial and the unblinded data on study endpoints and possible adverse effects to recommend continuation, alteration of study design, or early termination as appropriate. One reason for a premature termination might be that the DSMB comes to the conclusion that for the participants the potential risk to sustain adverse effects is greater than the potential benefit.

Should the Data Safety and Monitoring Board recommend that the trial is permanently discontinued prior to its planned completion, the investigators, the EC, and all other concerned parties will be appropriately notified of the rationale of such a decision. All study documentation, biological samples, and electronic data will be collected from the study cites and stored at the Coordinating Centre (Centre on Aging and Mobility, University of Zurich). All data will be stored safely and securely for the period required by theEU directive 2003/63/EC for at least 15 years.

All remaining supply of study interventions will be appropriately destroyed or returned to the producer. In case the study interventions are destroyed, an appropriate certificate of destruction will be issued and filed with the trial master file. We request explicitly from all participants that all data collected in case of drop-out can be used for analyses planned in DO-HEALTH and future analyses related to the DO-HEALTH biobank. It is important to have complete data on adverse effects and benefits of the interventions tested.

16.2. Planned Study Termination

The Data Safety and Monitoring Board, and the EC are notified of the study completion. All study documentation, and electronic data will be collected from the study cites and stored at the Coordinating Centre (Centre on Aging and Mobility, University of Zurich). The biological samples will be stored at Fisher Clinical Services GmbH (Allschwil, Switzerland). All data will be stored safely and securely for the period required by the EU Tissue and Cells Directive of for at least 15 years.

All remaining supply of study interventions will be appropriately destroyed or returned to the producer. In case the study interventions are destroyed, an appropriate certificate of destruction will be issued and filed with the trial master file.

17. Reporting, Publications, and Presentations

17.1. Routine Reporting to the European Commission

The European Commission will be kept updated on all aspects of the study progress in a timely manner. The following reports will be prepared and submitted for the review of the EC by the time points provided in the table. *Note: the timeline of deliverables will be updated after the amendment is approved by the EC.*

#	Deliverable Number	Title	Dissemination Level	Delivery Deadline (Months)
		5 Months		
1.	D1.2	DO-HEALTH registration at the International Trial Registry	Public	5
		9 Months		
2.	D5.17	CRFs and questionnaires are developed, checked for data consistency, and translated into all study languages	Confidential	9
		10 Months		
3.	D1.1	Study materials finalized	Confidential	6
4.	D2.10	Preparation for WP2 endpoints finalized	Confidential	6
5.	D3.11	Preparation for WP3 endpoints finalized	Confidential	6
6.	D4.12	Blood samples handling and operation manual	Public	6
7.	D7.26	Management preparation report	Confidential	6
8.	D8.31	Questionnaires on healthcare utilization are sent to data management	Confidential	6
		11 Months		
9.	D1.5	Global tasks of trial execution (T.1.8.1, intermediate recruitment goals)	Confidential	11
		18 Months		
10.	D9.39	DO-HEALTH intellectual property and exploration report, period 1	Confidential	18
		26 Months		
11.	D1.6	All baseline visits performed by all recruitment centers in 2152 seniors	Confidential	26
12.	D4.13	Baseline results for all biomarkers are send into the central data bank	Confidential	26

#	Deliverable Number	Title	Dissemination Level	Delivery Deadline (Months)
13.	D5.18	Baseline results of the central safety lab and baseline biobank report sent to the central data bank	Confidential	26
14.	D6.22	Baseline monitoring report	Confidential	26
15.	D7.27	Management report, period 1	Confidential	26
		34 Months		
16.	D1.7	All 12-month visits performed by all recruitment centers in 2152 seniors	Confidential	34
		36 Months		
17.	D9.40	DO-HEALTH intellectual property and exploration report, period 2	Confidential	36
		38 Months		
18.	D4.14	12-month results for all biomarkers are send into the central data bank	Restricted	38
19.	D5.19	12-month results of the central safety lab and baseline biobank report sent to the central data bank	Confidential	38
20.	D6.23	12-month monitoring report	Confidential	38
21.	D7.28	Management report, period 2	Confidential	38
		50 Months		
22.	D1.8	All 24-month visits performed by all recruitment centers in 2152 seniors	Confidential	50
23.	D4.15	24-month results for all biomarkers are send into the central data bank	Restricted	50
24.	D5.20	24-month results of the central safety lab and baseline biobank report sent to the central data bank	Confidential	50
25.	D6.24	24-month monitoring report	Confidential	50
26.	D7.29	Management report, period 3	Confidential	50
		54 Months		
27.	D9.41	DO-HEALTH intellectual property and exploration report, period 3	Confidential	54
		62 Months		
28.	D1.9	All 36-month visits performed by all recruitment centers in 2152 seniors	Confidential	62
29.	D4.16	36-month results for all biomarkers are send into the central data bank	Restricted	62

#	Deliverable Number	Title	Dissemination Level	Delivery Deadline (Months)
30.	D5.21	24-month results of the central safety lab and baseline biobank report sent to the central data bank	Confidential	62
31.	D6.25	36-month monitoring report	Confidential	62
32.	D7.30	Management report, period 4	Confidential	62
		66 Months		
33.	D8.33	Report on cost effectiveness	Confidential	66
34.	D9.38	DO-HEALTH reports disseminated	Public	66
35.	D9.41	DO-HEALTH intellectual property and exploration report, period 4	Confidential	66

17.2. Software and Models

The following software and statistical models will be developed during the course of DO-HEALTH.

#	Deliverable Number	Title	Dissemination Level	Delivery Deadline (Months)
1.	D9.34	DO-HEALTH website/communication	Public	10
2.	D1.3	Practical software for communication of DO-HEALTH findings (structural prototype)	Public	48
3.	D8.32	DO-HEALTH health economics model	Confidential	50
4.	D9.36	DO-HEALTH teaching software (structural prototype)	Confidential	50
5.	D9.37	DO-HEALTH teaching software (demo version)	Confidential	50
6.	D1.4	Practical software for communication of DO-HEALTH findings (final prototype)	Public	65
7.	D9.42	DO-HEALTH newsletters written	Public	66

17.3. Publication and Dissemination of Study Results

The high-quality trial design of the DO-HEALTH, sufficiently powered to test three promising public health interventions, supports scientific cooperation and provides the scientific basis for publications in high-level international "peer-reviewed" journals. Scientific performance and result dissemination is expected to proceed through the specialized and non-specialized scientific press. For public communication purposes, non-sensitive information will be shared on the public DO-HEALTH website.

All information from the DO-HEALTH trial design and results of the project (both baseline associations, which will create publication opportunities during the course of DO-HEALTH, and final results) will be available to the EU and to the world scientific community thus allowing a large diffusion of knowledge.

All communicated information shall always be controlled by the Intellectual Property Committee before publication or diffusion, so that in no way sensitive information provided by the partners is disclosed. Within the Plan for using and disseminating the foreground, the consortium will define a "roadmap" for exploitation and dissemination, and main project results will be identified regarding their (i) Health, healthcare, and clinical potentials; (ii) Scientific and technological impacts; (iii) Economic and industrial perspectives; and (iv) Community added value. The terms of IPR management will be specified in detail in the **Consortium Agreement** to be signed at the dawn of the project. They will be guided by **Annex II of the Model Contract**.

The following project meetings are planned to communicate information about DO-HEALTH to the authorities, scientific community, and general public. All project meetings are planned to take place at the University of Zurich.

Table 4. Planned project meetings

Type	Activity	Lead partner
Annual Project Meeting	Evaluation of scientific WPs based on overviews by WP leaders and scientific results by PhD students and post-docs	UZH: Year 1 and at the end
		CHARITE: Year 2
		IMU: Year 3
		CHUT: Year 4
Final Project Meeting	Evaluation of final results on overviews by WP leaders and scientific results by PhD students and Post-docs, as well as preparing for the final report	UZH
International final symposium	Present all results from DO-HEALTH	IOF

Table 5. Visits and assessments

Endpoints, procedures, assessments	Screening phone call	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Adverse events			X	X	X	X	X	X	X	X	X	X	X	X
Biomarkers[#]		X				X				X				X
Blood pressure		X				X				X				X
Body composition and fat mass (DXA)†		X				X				X				X
Bone mineral density in the spine and hip (DXA)[†]		X				X				X				X
Inclusion in the study (Mini Mental State Examination)		X												
Cognitive decline (MoCA; Montreal Cognitive Assessment)		X				X				X				X
Comorbidities		X				X				X				X
Demographic information	X[*]	X												
Disability regarding activities of daily living (PROMIS-HAQ)		X				X				X				X
Fall history		X												
Fasting blood glucose and insulin		X				X				X				X
Fracture history (non-vertebral, vertebral, hip)		X												
Frailty (SHARE Frailty Instrument)		X				X				X				X
Gait analyses and dual task assessment (GAITRite® Platinum)[‡]		X				X				X				X
Gastro-intestinal symptoms (Rome III questionnaire)		X				X				X				X
Grip strength		X				X				X				X
Height and weigth measurements		X				X				X				X
Hospital admissions			X	X	X	X	X	X	X	X	X	X	X	X

Endpoints, procedures, assessments	Screening phone call	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Implant infections after total hip or knee replacement			X	X	X	X	X	X	X	X	X	X	X	X
Incident cancer			X	X	X	X	X	X	X	X	X	X	X	X
Incident falls (any, low trauma, injurious)			X	X	X	X	X	X	X	X	X	X	X	X
Incident fractures (non-vertebral, vertebral and hip-) (DXA)[†]			X	X	X	X	X	X	X	X	X	X	X	X
Incident infections		X	X	X	X	X	X	X	X	X	X	X	X	X
Incident nephrolithiasis						X				X				X
Incident symptomatic osteoarthritis (knee, hip, and hand)		X				X				X				X
Informed consent		X												
Major cardiovascular events			X	X	X	X	X	X	X	X	X	X	X	X
Mental health decline, depression (GDS, *excerpt*)		X				X				X				X
Mortality			X	X	X	X	X	X	X	X	X	X	X	X
Muscle mass in the upper and lower extremities (DXA)[†]		X				X				X				X
Musculoskeletal pain (McGill pain map)		X				X				X				X
Nursing home admissions			X	X	X	X	X	X	X	X	X	X	X	X
Oral health		X				X				X				X
Physical activity		X				X				X				X
Physical examination		X				X				X				X
Pill count[##]						X				X				X
PUFA serum level		X				X				X				x
Quality of life (EQ5D-3L)		X		X		X		X		X		X		X
Randomization		X												

Endpoints, procedures, assessments	Screening phone call	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Self-reported adherence to study treatments and exercise program			X	X	X	X	X	X	X	X	X	X	X	X
Serum 25(OH)D		X				X				X				X
Serum albumin		X				X				X				X
Serum calcium		X				X				X				X
Serum creatinine		X				X				X				X
Serum phosphate		X				X				X				X
Blood count (baseline assessment)		X												
Short Physical Performance Battery (including reaction time)		X				X				X				X
Symptomatic osteoarthritis (severity of knee pain, knee buckling, total number of joints with pain, and NSAID use)		X				X				X				X
Tooth loss		X				X				X				X
Diet assessment by Food Frequency Questionnaire		X												X
Fracture healing of incident fractures[++]														

[*] Some information that is needed to screen prospective participants is collected, however the information is destroyed after eligibility for baseline visit is determined.

[†] Measurement taken in 1502 participants at four study centers equipped with iDXA machines.

[‡] Measurement taken in 250 seniors recruited at Basel University Hospital.

[#] TNF-α, IL10, IL-17, 1L-22, CD3, CD4, CD25, CD127 will be measured in 802 participants only.

[++] Will be assessed only in individuals with incident major osteoporotic fractures at the arm (shoulder, humerus, forearm) and leg (hip, femur, ankle): (a) *primary fracture healing endpoint*: clinical fracture healing with 3 additional phone calls at 6, 12, 18 weeks after the fracture assessing the PROMIS-HAQ (b) *secondary fracture healing endpoint*: observed functional fracture healing measured with the Short Physical Performance Test Battery and grip strength at regular 12, 24, 36 month visits (c) *exploratory fracture healing endpoint*: radiological fracture healing with independent assessment of early (6-8 weeks) and late (12 to 14 weeks) consolidation assessment based on standard x-rays.

##Pill count will also be performed outside clinical visits when required.

Table 6. Questionnaires

Questionnaire	Screening phone call	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
EQ5D-3L		X		X		X		X		X		X		X
Fall questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X
GDS *(excerpt)*		X				X				X				X
GOHAI		X				X				X				X
HOOS		X				X				X				X
Infection questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X
Joint map		X				X				X				X
KOOS		X				X				X				X
Mc Gill map (modified version)		X				X				X				X
MoCA (Montreal Cognitive Assessment)		X				X				X				X
NHS, excerpt		X				X				X				X
PROMIS-HAQ		X				X				X				X
*Quick*DASH		X				X				X				X
ROME III		X				X				X				X
SHARE-FI		X				X				X				X
Diet assessment by Food Frequency Questionnaire		X												X
Fracture healing of incident fractures[++]														

[++]Will be assessed only in individuals with incident major osteoporotic fractures at the arm (shoulder, humerus, forearm) and leg (hip, femur, ankle): (a) *primary fracture healing endpoint*: clinical fracture healing with 3 additional phone calls at 6, 12, 18 weeks after the fracture assessing the PROMIS-HAQ (b) *secondary fracture healing endpoint*: observed functional fracture healing measured with the Short Physical Performance Test Battery and grip strength at regular 12, 24, 36 month visits (c) *exploratory fracture healing endpoint*: radiological fracture healing with independent assessment of early (6-8 weeks) and late (12 to 14 weeks) consolidation assessment based on standard x-rays.

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Appendix

DO-HEALTH Safety & Applicability

Elderly volunteers will consume daily food supplements containing 1,000 mg EPA/DHA (algal oil) and 2,000 IU (50 µg) vitamin D_3.

I. Algal oil at 1000 mg/ day of combined EPA/DHA

Oils derived from marine organisms are currently the only source of long-chain polyunsaturated omega-3s known as EPA and DHA. Such oils are historically obtained in the human diet via the consumption of fish. More recently, purified oils are available in the form of food supplements; such oils are extracted from fish, most usually anchovy and sardine, caught off the coast of South America.

EPA and DHA accumulate in the marine food chain, and the base of this food chain is primary production within microscopic algae. During the 1980s, methods were developed to cultivate selected algae in large enclosed fermentation, and products were commercialized around the world in mid 1990s. This industry was pioneered by a limited number of companies; all of the products marketed are closely related (with some variation in the strain of algae and resultant fatty acid ratios).

These 'algal oil' products are high in selected fatty acids and consistent in quality. They are widely used in the food chain, in particular within infant nutrition. For example, long-chain polyunsaturated omega-3 used in infant formula in the US is all derived from algal fermentation.

Algal oils have less penetration into other applications such as food supplements due to their high cost compared with fish oils. Notwithstanding, such supplements are widely available and are popular with some consumers such as vegetarians, consumers concerned about marine pollution and less price-sensitive individuals.

Since the mid-1990s many variations of algal oil have been successfully assessed by US and European food authorities (see Table 1). Due to the available efficacy data, most supplements deliver more than 250 mg/ day and up to 2,000 mg/ day of EPA and DHA. These levels of intake are in line with that achieved by individuals who habitually consume fresh oily fish. EPA- and DHA- rich oil derived from algal fermentation is therefore a regular food ingredient, with 1,000 mg/ day of EPA and DHA being a normal and healthy intake level.

The oil intended to be used in DO-HEALTH has been positively assessed under the US regulatory system (known as 'GRAS'), and has recently had a positive opinion from the UK Authority. The UK assessment can be viewed at the following link, which also provides comprehensive information for the oil: http://acnfp.food.gov.uk/assess/fullapplics/dhanadeparichalgaloil/

Table 7: Examples of authority assessments of DHA-rich oil produced from algae in fermentation

Authority	Manufacturer	Application	Approval Year
Food Standards Australia & New Zealand	Omega-Tech	Infant	2002
	Martek	General Food	2003
	Lonza	General Food	2005
EU Member States / EU Food Safety Authority	Omega-Tech	Infant	1995
	Martek	General Food	2002
	Lonza	General Food	2003
US Food & Drug Administration	Monsanto	Food Supplements	1997
	Omega-Tech	Food Supplements	2002
	Martek	Infant	2001
	Martek	General Food	2010
Health Canada	Martek	Infant	2002
	Martek	General Food	2006

II. Vitamin D3 at 2,000 IU (50 µg/day)

Vitamin D_3 is a pro-hormone that regulates a large number of genes; it is synthesized in the skin and is available in very limited quantities from the diet. Individuals who habitually expose the majority of their skin surface area to sunshine at a latitude historically associated with their pigmentation type (e.g. fair skinned in the north, darker skinned in the south) achieve a production of vitamin D_3 equivalent to consumption of 10,000 IU (250 µg) per day [(1,2)]. This level of production, known as the physiological maximum, is also the level that has been determined to be the safe upper limit on the basis of review of human evidence [(3)].

The approaches historically applied to determining both the recommended intake and safe upper levels of vitamin D_3 have resulted in quantities that are too low to satisfy health promotion. This situation is currently being rectified around the world by different authorities.

The classical approach to determine vitamin requirement relied on clinical observations of gross effects resulting from deficiency, and the amount of vitamin required in order to alleviate the deficiency. Therefore the levels have been set based on preventing only the most severe effects, rather than providing maximum health benefit related to less easily observed effects. In the case of vitamin D, historical intake levels are related to preventing gross malformations of the bones, such observations are easily observed by clinicians. However, it is now known that the intake required to promote healthy bone density and structure is higher than that required to prevent gross malformations [(7)].

This situation is exacerbated with respect to vitamin D_3, as the mechanism of action is via the regulation of many genes. It is now proven and validated by the European Food Safety Authority that vitamin D_3

has a key role in regulating immune function, muscle function (including prevention of falling in the elderly), and in the control of normal cell division [4-6].

There is also a problem with the historical methods used to assess the safe upper levels of vitamins. Classical toxicology, as applied to chemicals has traditionally been used. In this approach a 'safe' level is determined from a pivotal study in which the adverse effect is also demonstrated (such as hypercalcaemia), and then arbitrary 'safety factors' are applied such that a conservative safe level for humans is set.

This classical approach has led to unrealistically conservative estimates of safe upper intake. As discussed above, deriving a safe level based only on an analysis of human study data has led to far higher estimations, furthermore, the natural physiological level from dermal production is known.

Therefore, recommended intakes and safe upper levels are now being reviewed worldwide taking into account modern data and approaches. Notably, the US Institute of Medicine (upon whose opinion US policy is set) has recently established the upper safe level at 4,000 IU (100 µg) per day for all age/gender groups above 8 years old [8]. Other authorities, including within the EU, are currently conducting their evaluations, and it is likely that these will set the same or similar level.

The delay in updating regulations on vitamin D is exacerbated within Europe as there is currently no consistent approach to upper levels between member states. The current situation is difficult for national authorities to manage, in many cases the approach adopted is to officially quote conservative levels based on the historical methods, but as it is accepted that these levels are too low; products that contain higher levels are not excluded from the market. Therefore, food supplements that provide 2,000 IU (50µg)/ day of vitamin D_3 are commonly retailed across the EU market.

III. Conclusions

Modern lifestyles and eating patterns have significantly diverged from those in which the human species evolved. In particular, the intake of long-chain omega-3 and production of vitamin D_3 is comparatively limited in the modern era. The health impacts of this limitation are now well documented and recognized by authorities and the slow process of updating official recommendations is ongoing.

Products that provide 1000 mg/day EPA & DHA from algal fermentation and 2,000 IU (50µg)/ day of vitamin D_3 are commercially available in the food chain, and increasingly popular within Europe. Improving the knowledge base on the benefit achieved by taking adequate levels of these nutrients is essential in promoting health, particularly so in the ageing population.

IV. References

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Signature Page

Vitamin D3 – Omega3 – Home Exercise – HeALTHy Ageing and Longevity Trial

Randomized, double-blind, placebo-controlled, multi-centre clinical trial

Version 3.0 dated March 3, 2015

Clinical Investigation Plan Approval Signature

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Table of Contents

1. Abbreviations and Definitions

ACR - American College of Rheumatology
ALT – Alanine Aminotransferase
AST – Asparagine Aminotransferase
BMD – Bone Mineral Density
CoV – Coefficient of Variability
CRF – Case Report Form
CRP – C-reactive Protein
DHA – Docosahexaenoic Acid
DSMB – Data Safety and Monitoring Board
DXA or iDXA – Dual-energy X-ray Absorptiometry
EPA – Eicosapentaenoic Acid
FRAX® – WHO Fracture Risk Assessment Tool
GEE – Generalized Estimating Equation
GI – Gastro-intestinal
HDL – High Density Lipoprotein
HOOS – Hip injury and Osteoarthritis Outcome Score
Incidence– means that an individual has the event or not
ITT – Intention to Treat
KOOS – Knee injury and Osteoarthritis Outcome Score
LDH – Lactate Dehydrogenase
MAR – Missing at Random
MMSE – Mini Mental State Examination
Number of – means that a person can have multiple events. This outcome counts a total number of events experienced by a participant.
OA – Osteoarthritis
PI – Principal Investigator
PUFA – Polyunsaturated Fatty Acid
SD – Standard Deviation
SPPB – Short Physical Performance Battery
TSH – Thyroid Stimulating Hormone

2. Introduction

2.1. Preface

As the European population is ageing rapidly, the growing number of seniors with age-related chronic diseases poses a challenge on European societies and health care systems. Therapeutic interventions that are effective, affordable, and well-tolerated in the prevention of chronic disease are urgently needed and will have an outstanding impact on public health as a whole. Among the most promising interventions that meet these requirements are vitamin D, marine omega-3 fatty acids and physical exercise. However, their individual and combined effects have yet to be confirmed in a clinical trial. DO-HEALTH will close this knowledge gap in a large 3-year multi-center clinical trial that will establish long-term efficacy and safety data for the 3 interventions in the prevention of age-related diseases in seniors.

DO-HEALTHwill enroll 2152 community-dwelling men and women who are 70 years and older, an age when chronic diseases increase substantially. DO-HEALTH will test the individual and the combined benefit of 2000 IU vitamin D/day, 1 g of omega-3 fatty acids/day and a simple home exercise program in an efficient randomized-controlled factorial design trial. The trial will define the role of the 3 interventions in the prevention of 5primary endpoints: the risk of incident non-vertebral fractures; the risk of functional decline; the risk of blood pressure increase; the risk of cognitive decline; and the rate of any infection. Key secondary endpoints include risk of hip fracture, rate of falls, pain in symptomatic knee osteoarthritis, gastro-intestinal symptoms, mental and oral health, quality of life, and mortality. Follow-up will be in-person, in 3-monthly intervals (4 clinical visits and 9 phone calls). DO-HEALTH will further assess the comparative effectiveness of the interventions by evaluating reasons why or why not seniors adhere to them, and will assess their cost-benefit in a health economic model based on documented health care utilization and observed incidence of chronic disease.

2.2. Purpose of the analyses

These analyses will assess the efficacy and safety of daily supplementation with vitamin D_3 (2000 IU/day vs. placebo), Omega-3 fatty acids (Eicosapentaenoic Acid[EPA] +Docosahexaenoic Acid[DHA], 1g/day vs. placebo), and home-basedexercise program (muscle strength exercise [intervention] vs. joint mobility exercise [control]). Study treatments will be compared alone and in combination and the results will be included in the clinical trial report.

3. Study Objectives and Endpoints

3.1. Study Objectives

The aim of this multi-center, randomized, double-blind, placebo-controlled, 2×2×2 factorial clinical trial (n = 2152; 269/treatment group) is to assess the role of vitamin D_3 (2000 IU/day), and/ or omega-3 fatty acids (1 g/day EPA + DHA, ratio 2:1), and/ora simple home-based exercise program (30 min. × 3 times per week) vs. control over 36 months in prevention of chronic disease in seniors aged 70 and olderand contribution of the interventions to healthy ageing.

- To test whether 2000 IU/day of vitamin D_3 reduces risk of chronic disease in seniors compared to placebo
- To test whether 1 g/day of marine omega-3 fatty acids (EPA+DHA) reduces the risk of chronic disease in seniors compared to placebo
- To test whether a simple home "muscle strength" exercise program reduces the risk of chronic disease in seniors compared to control ("joint mobility" exercise) performed 30 minutes 3 times a week)
- To test whether there is an additive value of the 3 interventions combined as a multi-modal intervention in the reduction of chronic disease in seniors
- To assess the comparative effectiveness of the interventions and to test whether and to what degree adherence modulates the effect of the 3 interventions on risk reduction of chronic disease in seniors
- To test whether subgroups of the senior population (by gender, age [70-84; 85+], body mass index, baseline physical activity, baseline serum 25(OH)D levels, baseline PUFA levels, previous fall (in the year preceding enrollment in the DO-HEALTH trial), previous fracture (last 10 years), FRAX – estimated absolute fracture risk, baseline clinical knee OA, and baseline calcium and protein intake) have a differential benefit from the 3 interventions regarding risk reduction of chronic disease
- To assess the cost-benefit of the 3 interventions individually and in combination as a multi-modal intervention based on an objective health economic model
- To improve medical care of seniors by establishing laboratory reference ranges for a large set of common laboratory markers and by extending the WHO FRAX fracture prediction model by including the risk of falling
- To validate novel biomarkers of bone and muscle functionality and immunity based on their response to the 3 interventions and based on the incidence of musculoskeletal and immunity endpoints

3.2. Endpoints

3.2.1. Primary Endpoints

Bone:Incidenceof non-vertebral fractures (Only 1 fracture per person). All fracture events are confirmed by X-ray reports or medical records that describe an X-ray report of the fracture or mention the repair of the fracture. The endpoint is assessed every 3 month (4 visits and 9 phone calls) in all 2152 seniors. (See DO-HEALTH fracture assessment manual for details of fracture assessment).

Muscle:Functional declineis assessed by aShort Physical Performance Battery (SPPB) score.SPPB is an objective assessment tool for evaluating lower extremity function in older persons(1). The SPPB score ranges from 0 (poor) to 12 (good) and is assessed at baseline, 12, 24, and 36 months in all 2152 participants.

Cardiovascular:Change in systolic and diastolic blood pressure. Blood pressure ismeasuredin all 2152 seniors at baseline, 12, 24, and 36 monthsafter 5-minute rest in seated position following a standardized protocol validated in DO-HEALTH pilot trials(2).

Brain:Cognitive decline is assessed by aMontreal Cognitive Assessment Tool (MoCA) score(3).The MoCA score assesses cognitive function and ranges from 0 (severe cognitive function impairment) to 30 (normal cognitive function).The score is measured at baseline, 12, 24, and 36 months in all 2152 seniors.

Immunity:Number of infections of any type. Incidence of infections is assessed every 3 months (4 clinical visits and 9 phone calls) in all 2152 seniors by a detailed infection questionnaire developed in two pilot trials to DO-HEALTH (4, 5). The questionnaire collects information about symptoms, treatment, MD contact and medical assessments. Every case of infection must be confirmed by medical records.

3.2.2. Secondary Endpoints

Bone

Incidenceof hip fractures. Hip fractures include femoral neck and trochanteric fractures. Incident hip fractures are recorded every 3 month (4 visits and 9 phone calls) in all 2152 seniors.Diagnosis of a hip fracture is confirmed by X-ray reports or medical records mentioning an X-ray report or fracture repair(See DO-HEALTH fracture assessment manual for details of fracture assessment).

Incidenceof vertebral fractures. Incident vertebral fractures are recorded at baseline, 12, 24, and 36 months in 1502 participants at 5 recruiting centers equipped with dual-energy x-ray absorptiometry(iDXA) machines (Zurich, Berlin, Toulouse, Nurnberg, and Coimbra). Diagnosis of a vertebral fracture isbased on iDXA vertebral morphometry assessment that is performed centrally at a specialized center (Charité, Felsenberg) by personnel blinded to treatment assignments. Once diagnosed,the vertebral deformities due to osteoporosis are classified as eithermild to severe ormoderate to severe deformities. Incidence of vertebral deformities of each category is recorded separately.

Incidenceof anyfractures. This endpoint includesany new non-vertebral fracture (see primary endpoint) PLUS any new vertebral fracture (mild to severe deformity based on vertebral morphometry). The endpoint is assessed in 1502 seniors with iDXA measurements over 36-month period.

Bone mineral density (BMD)at the spine and hip. BMD is measured by iDXAat baseline, 12, 24, and 36 months in 1502 participants at 5 recruiting centers equipped with iDXA machines.

Muscle

Number ofany low trauma falls.A fall is defined as unintentional coming to rest on the ground, floor, or other lower level (coming to rest against furniture or a wall is not considered a fall).Low trauma fall is defined as a fall occurring from a standing height and without the involvement of others or a vehicle. Incident low trauma falls are ascertained with DO-HEALTH fall questionnaireinall 2152 participants every 3 months (4 visits and 9 phone calls).

Numberof injurious falls. An injurious fall is defined as a low trauma fall that leads to any injury (i.e. skin wound, significant bruising, or fracture). Incident injurious falls are ascertained with DO-HEALTH fall questionnaire in all 2152 participants every 3 months (4 visits and 9 phone calls).

Number of participants who fell. The endpoint is assessed in all 2152 participants every 3 months (4 visits and 9 phone calls).Any participant who reported at least 1 low trauma fall during a 3-month period is defined as a person who fell during that period; if multiple falls occurred to one person during a period, the person is counted once for this endpoint.

Reaction time. Reaction time is assessed by the repeated chair stands test (a part of the SPPB(1))in all 2152 participants at baseline, 12, 24, and 36 months.The test times 5 repeated stand ups from a chair.

Grip strength. Grip strength is assessed with validated grip strength protocol using Martin vigorimeter(6) in all 2152 participants at baseline, 12, 24, and 36 months.

Muscle mass in the upper and lower extremities. Muscle massis measured visits at baseline, 12, 24, and 36 months by iDXA in 1502 participants at 5 recruiting centers equipped with iDXA machines.

Musculoskeletal pain. Musculoskeletal painis assessed using McGill pain map (7)in all 2152 participants at baseline, 12, 24, and 36 months.

Commented [ES1]: If the outcome is the number of painful regions, this may need repeated measures Poisson.

Dual tasking gait speed. Dual tasking gait speedis assessed by comparing normal gait speed against gait speed when a simple cognitive task is being performed at the same time (8). Participants are timed walking a 10 meter course 2 times. First they walk at their normal pace and then they walk normally simultaneously performing a simple cognitive task. The gait speed is calculated as V = (10 meters/time, sec).The difference between the two times is evaluated for this endpoint. The endpoint is assessed in all 2152 participants at baseline, 12, 24, 36 months.

Cardiovascular

Incidence of hypertension over 36 month. Blood pressure is measured after a 5-minute rest in a seated position following a standardized protocol validated in one of the DO-HEALTH pilot trials (9). Measurements are taken at baseline, 12, 24, and 36 monthsin all 2152 participants and each case (person) of incident hypertension (blood pressure of 140/90 mmHg or higher) that occurred over the 36-month follow-up period isrecorded. In addition, a new chart-based diagnosis of incident hypertension and/or a report of new use of anti-hypertensive drugs is counted as a case of incident hypertension (person). Each case of incident hypertension is confirmedeither (1) based on blood pressure measurement taken during the clinical visits, or (2) a new diagnosis by a primary care physician or report of a new use of anti-hypertensive drug documentedin medical records. All cases of incident hypertension are confirmed or disconfirmed by the Independent Physician Endpoint Committee.

Brain

Mental health declinewill be assessed at baseline, 12, 24, and 36 months in all 2152 participants using the Montreal Cognitive Assessment (MoCA) score.This outcome will be defined by a binary and a continuous variable. The binary variable will be defined as decline in MoCA score compared with baseline (yes/no). The continuous variable will be the actual decline in MoCA score compared with baseline.

Commented [ES2]: This definition has been updated. Is it OK like this?

Incidence of depressionis assessed at baseline, 12, 24, and 36 months in all 2152 participants usingscores from the Geriatric Depression ScaleShort Form (GDS-SF). The scale defines scores of≤ 5 as normal, 5 – 9 as suggestive depression, and 10+ as depression. For this outcome, depression is defined as having GDS-SF score of 10 or more.

Dual tasking gait variability is assessedby gait analyses and dual task assessments using GAITRite® Platinum gait analysis system(CIR Systems, PA, USA)in 250 participants recruited atBasel University Hospital at baseline, 12, 24, and 36 months. Seniors will perform 5 walking tasks with and without cognitive challenge. The endpoint is recorded as mean, standard deviation (SD), and coefficient of variability (CoV = [SD/mean] × 100) of stride time and stride velocity(10).

Commented [ES3]: The protocol mentions 4 tasks, but in the GAITRite CRF for Basel we have 5: single task walk in normal, fast, and slow pace, working memory task, and semantic memory task (the latter two are dual-task tests). This set of tests was proposed by the Basel team. I think if we have the 5 tests we might as well use them all.

Commented [ES4]: Is this correct? These parameters were evaluated in the paper referenced here.

Immunity

Number of any upper respiratory infections,flu-likeillnesses, and severe infections that lead to hospital admission over the 36-month period. Incident infections are assessed every 3 months (4 clinical visits and 9 phone calls) in all 2152 seniors by a detailed infection questionnaire (see **Immunity** primary endpoint above). Infection typeis confirmed by the Independent Physician Endpoint Committee. Cases of infections that lead to hospital admission are confirmed by medical records.The endpoint is defined as total number of incident upper respiratory infections, flu-like illnesses, and severe infections that lead to hospital admission that each participant experienced until the end of follow up or drop out.

Bone/cartilage: the following two endpoints will be analyzed in the subgroup of patients with symptomatic knee OA at baseline. All 2152 participants are evaluated for prevalent knee OA at baseline clinical visit. Knee OA is defined according to the American College of Rheumatology (ACR) criteria (11).

Severity of knee painis assessed withthe Knee injury and Osteoarthritis Outcome Score (KOOS (12)) at baseline, 12, 24, and 36 months. KOOS Pain scale score is a normalized score ranging from 100 (no symptoms) to 0 (extreme pain) is used to assess pain in all participants. This outcome is defined as a KOOS score scored by a participant at each time point.

Rate of knee buckling. Knee buckling is assessed at baseline, 12, 24, and 36 months with Knee Buckling Questionnaire (Felson et al., 2008).The endpoint is assessed by question 3 of the Knee Buckling Questionnaire. The question has 5 categories of answers evaluating the number of time participant's knees buckled during the past 3 months: once, 2 – 5, 6 – 10, 11 – 24, and 24+ times. This endpoint is analyzed as a categorical variable.

Bone/cartilage: the following two endpoints will be analyzed in all participants:

Total number of joints with pain. Total number of joints with pain is recorded in all 2152 at baseline, 12, 24, and 36 months with Joint Map (homunculus). Total number of painful joints indicated by a participant on the map at each time pointisrecorded for this endpoint.

NSAID use due to knee pain is recorded from the Concomitant Medication section of the Case Report Form (CRF)in all 2152 at baseline, 12, 24, and 36 months. This endpoint is defined as a binary (yes/no) variable at each time point.

Dental

Decline in oral healthis assessed in all 2152 participants at baseline, 12, 24, and 36 months with the Geriatric Oral Health Assessment Index (GOHAI(13)) questionnaire.GOHAI instrument consists of 12 Likert-scale questions. A participant can score from 0 to 5 on each of the question and the final GOHAI score is determined by summing the scores for individual questions. The final score ranges from 0 (worst oral health) to 60 (best oral health).

Tooth lossis assessed in all 2152 participants every 3 months (4 clinical visits and 9 phone calls). At each contact (personal of phone) participants report whether they have lost teeth during the period since last contact, and, if yes, how many teeth were lost. At each clinical visit(baseline, 12, 24, and 36 months) teeth are counted by the study MD. Total number of teeth (own and prosthetic) is recorded as well as the number of own, missing, and prosthetic (removable and fixed) teeth. Thetooth counts are centrally validatedat the University of Zurich using tooth prints.

Gastro-intestinal

Number of any newgastro-intestinal symptoms is assessed in all 2152 participants at baseline, 12, 24, and 36 months using ROME III diagnostic questionnaire for the adult functional gastro-intestinal (GI) disorders(14).

Glucose-metabolic

Fasting blood concentration of glucose and insulinis measured in all 2152 participants at baseline, 12, 24, and 36 months. Insulin sensitivity and beta cell function derived from indices of fasting glucose and insulin concentrations are calculated using quantitative insulin-sensitivity check index (QUICKI), and the HOMA index(15).

Body compositionand fat massare measured by iDXAduring clinical visits at baseline, 12, 24, and 36 months in 1502 participants recruited by 5centers equipped with iDXA machines. Trunk and upper and lower extremities fat mass is assessed for this endpoint.For this endpoint we are going to use percentage and mass of fat tissue and percentage and mass of lean muscle. These measurements are measured directly by DXA.

Commented [ES5]: This endpoint may be analyzed by a separate Poisson model for each of the time points and also by a repeated measures Poisson model that includes information for all time points.

Commented [ES6]: No information on how GOHAI will be scored is provided at this point.
Heike: Please look at the publication and then we discuss

Commented [ES7]: I think the scale is wide enough for the score, so I propose we analyze it with a linear mixed model.

Commented [ES8]: May need repeated measures Poisson

John: agreed – should be addressed later

Commented [ES9]: Is that % of fat, muscle and bone in the body?

Heike: Please look at publications and make some suggestions to discuss

Kidney

Decline in kidney function. Kidney function is evaluated using serum creatinine level and glomerular filtration rate.Blood creatinine levels is measured in all 2152 participants at baseline, 12, 24, and 36 monthsand glomerular filtration rateis estimated with the Cockcroft-Gault formula(16).

Global Health

Quality of lifeis evaluated using the EQ5D-3L questionnaire in all 2152 participants at baseline, and every 6 months afterwards (during 6-, 18-, and 30-month phone calls, and at 12-, 24-, and 36-month clinical visits). A summary EQ index will be calculated for this endpoint. The index will be based on the time trade-off valuation technique and will be calculated using country-specific algorithms provided by EuroQol Executive Office (userinformationservice@euroqol.org). In case an algorithm is not available for a particular country where a study center is located, the algorithm for the country that is closest culturally to that territory will be used. We will used the following country-specific algorithms for DO-HEALTH study centers: Zurich, Basel, Innsbruck, and Berlin – a TTO algorithm for Germany; Geneva and Toulouse – a TTO algorithm for France; Coimbra – a TTO algorithm for Spain.

Frailty level. Frailtyis assessed withthe Survey of Health, Ageing and Retirement in Europe Frailty Instrument (SHARE-FI) (17) in all 2152 participants at baseline, 12, 24, and 36 months.Frailty scores will be calculated using gender-specific DFactor score models proposed by Romero-Ortuno et al. (17). For this endpoint we will run a GEE model to compare proportions of frail seniors among the treatment groups at eachtime point.

Level of activities of daily living is measured by the PROMIS-HAQ at baseline, 12, 24, and 36 months(18)**.** For this endpoint we will calculate HAQ score as described in the scoring instructions published by Stanford University School of Medicine (19).The calculated score ranges from 0 (absence of disability) to 100 (maximum disability) and will be analyzed as a continuous score.~~The endpoint is defined as havingat least one new limitation in 20 activities of daily living.Limitations are defined at two levels: some difficulty to do the activity and inability to do the activity.~~

Incident mortality, incident permanent nursing home admissions, andthe numberof acute hospital admissions are assessed every 3 months(4 clinical visits and 9 phone calls) in all 2152 study participants. Admissions to a nursing home or a hospital are confirmed by medical records. Mortality is confirmed by medical records or death certificate; cause of death is established from medical records or death certificate and classified as cardiovascular, cancer, severe infections, or other.

Commented [HB10]: Look at the score please

Commented [ES11]: What level do we use to define a limitation?

Heike: please look at the questionnaire and discuss

Commented [pchoca12R11]: I did a quick literature review, and could not find a cutoff point. Continuous is good.

3.2.3. Biomarker Endpoints

The following biomarkers are measured in blood or urine of all 2152 trial participants at baseline, 12, 24, and 36 months of follow-up. All measurements will be performed centrally by the clinical laboratory at Zurich University Hospital. In the analysis all measurements will be treated as continuous variables.

Bone:calcium, phosphate, 25(OH)D, intact PTH,urinary calcium/creatinine ratio (second spot urine), Beta-Crosslaps serum, P1NP, sclerostin

Muscle: myostatin

Cardiovascular: troponin T, NT-proBNP, homocysteine, CK, LDH, cholesterol, HDL-cholesterol, triglycerides

Inflammation: CRP, high sensitivity-CRP, IL6

Gastro-intestinal: ALT, AST, gGT, alkaline phosphatase, bilirubin

Glucose-metabolic: fasting glucose, insulin

Kidney: serum creatinine, calcium/creatinine ratio in second spot urine, serum urea, uric acid

Global Health:
Ions: sodium, potassium, chloride, magnesium
Proteins: total protein, albumin, ferritin, transferrin
Hormones: TSH, fT4, fT3, cortisol
Vitamins: folic acid, vitamin B12, 25(OH)D

Adherence to treatment: serum 25(OH)D, plasma PUFA concentrations (EPA, AA, DPA, DHA).

The following biomarkers are measured in 700 participants from three study centers (Zurich, Basel, Geneva) at baseline, 12, 24, and 36 months.

Inflammation: TNF-α, IL10, IL-17, IL-22.

Cellular immunity:CD3, CD4, CD25, CD127~~number of CD16/56-positive natural killer cells and the number of regulatory T cells~~.

3.2.4. Exploratory Endpoints

Bone

Incidence ofrepeat fracturesis defined as the occurrence of a second incident fracture occurring in the same individual at two different time points. Multiple fractures that occur at the same time point are not counted as repeat fractures. Any non-vertebral repeat fracture is evaluated in all participants (2152 participants), while vertebral fractures and total fractures are evaluated only among participants with iDXA measurements (1502 participants). All fracture events are confirmed by X-ray reports or medical records that describe an X-ray report of the fracture or mention the repair of the fracture.

Muscle

Incident disability regarding activities of daily living is measured by the PROMIS-HAQ at baseline, 12, 24, and 36 months(18)**.** ~~The endpoint is defined as havingat least one new limitation in 20 activities of daily living. In our first set of~~ analyses, limitations will be defined as having either some difficulty to do the activity or inability to do the activity. ~~In our second set of analyses, limitations will be defined as inability to do the activity.~~ The calculated 0 -100 HAQ score (see "Level of activities of daily living"above for details) will be converted into a binary variable: maintained or improved disability level over 36 months vs. disability level has worsened over 36 months.For this endpoint only the first incidence of worsening the disability level will be counted, i.e.: if the disability level stayed the same at 12 months, worsened at 24 months and worsened still at 36 months, the endpoint will be recorded as the disability

Commented [ES13]: What level do we use to define a limitation?

Heike: please look at the questionnaire and discuss

level had worsened at 24 months. We will analyze: 1) incident disability over 36 months (logistic regression model) and 2) time until incident disability (Cox survival model).

Incident sarcopenia. Since there is no consensus on a universally accepted definition of sarcopenia to date, all previously used and newly proposed composite definitions of sarcopenia based on appendicular muscle mass and the SPPB, its components(20) and/or grip strength, will be considered. Sarcopenia definition variables for this endpoint are derived from muscle mass, SPPB, and grip strength measurements(see Primary Endpoints and Secondary Endpoints sections above for details) performed in 1502 participants with iDXA measurements at baseline, 12, 24, and 36 months.

Incident frailty will be assessed with the Linda Fried Criteria

Fried / Hopkins Criterion	Assessing CRF
Unintentional weight loss: Unintentional loss of 10 lb. (4.5 kg) or more in the previous year at baseline (there is no previous weight measurement available) or loss of 5% of body weight or more during a year at follow up (previous year weight measurement is available).	Baseline weight: F102, item 5 Follow up weight: F304 We do not ask the participants whether they were trying to lose weight or not (the Fried criterion talks about unintentional weight loss). We can either add this question to the form or use section 37 of FFQ. The question in this section asks: "Are you currently following a special diet?" if the answer is "yes", a participant is asked to indicate reasons for the diet and one of the reasons is "Weight loss". If this is indicated, we know that the person was actively trying to loose weight. The caveat, of course, is that a person may choose exercise to loose weight, but I think that nowadays they usually recommend both diet and exercise (and diet is more intuitive and easier to do). I believe that it would be pretty rare that a participant would exercise for weight loss, but not be on a diet, so FFQ section 37 should work.
Exhaustion: This criterion uses 2 questions from the CES-D depression scale: a) *I felt everything I did was an effort* and b) *I could not get going*. These questions have 4 answers: 0 = rarely or none of the time (,1 day), 1 = some or a little of the time (1–2 days), 2 = a moderate amount of the time (3–4 days), or 3 = most of the time. Participants answering "2" or	In DO-HEALTH we are using an excerpt from GDS. There are no question in GDS that is directly equivalent to those CES-D questions needed for this criterion. Besides, GDS and CES-D response structures are different: GDS is a series of yes/no questions whereas CES-D has a scale of responses measuring frequency during last week: <1 day, 1 – 2 days, 3 – 4 days, 5 – 7 days. Alternatives:

Commented [ES14]: The alternative would be to categorize the variable as no disability (score of 0) vs any disability, but than we would have to exclude those with prevalent disability (score > 0 at baseline) limiting our sample size. I also think that in our age group we will not have many with score 0. This would require a person to perform all 20 tasks with no difficulties whatsoever.

Commented [ES15]: Spell out cutoffs for the Fried criteria and spell out how the score is calculated.
Is there normalized data for weight loss in Europe to adapt frailty criteria for baseline
Grip strength cutoff criteria (Linda Fried some peculiar way of doing this)

Commented [HB16]: Eduard – please check if we assess all criteria – may come from different corners of our data assessment – please check and discuss

Commented [ES17]: We can get 4 out of 5 criteria. The only problematic one is the Exhaustion one. I have put together a table summarizing the criteria and where and how they are assessed in DO-HEALTH. The table is for informational purposes only, I do not plan to keep it in the final version it is there just so we have everything in one place.

<table>
<tr>
<td>"3" to either of these questions are categorized as frail by the exhaustion criterion.</td>
<td>We could take the approach proposed byRomero-Ortuno et al. (2010) and Santos-Eggimann et al. (2009). They use SHARE-FI question 1: "In the last month, have you had too little energy to do things you wanted to?" They considered anyone who answered "Yes" to this question as those fulfilled the exhaustion criterion. The paper by Santos-Eggimann talks about adaptation of SHARE survey data to Fried criteria and the paper by Romero-Ortuno is using this method and defines the variables the same way for their analysis.</td>
</tr>
<tr>
<td><u>Physical activity:</u>

Men < 383 Kcal/week.

Women < 270 Kcal/week</td>
<td>We use NHS questionnaire to access physical activity. Although it is not the same questionnaire as the one used by Fried et al., it collects details about physical activities. We can use Compendium of Physical Activities (https://sites.google.com/site/compendiumofphysicalactivities/) to estimate energy expenditure. The compendium gives energy expenditure in METs, but since $1MET = \frac{1Kcal}{1kg \times 1h}$, we can convert METs to Kcal: 1Kcal = 1MET × 1kg × 1h.</td>
</tr>
<tr>
<td><u>Walk time:</u>

Distance – 15 feet (4.572 m)

Cutoffs:

Men

<u>Height</u> <u>Cutoff time</u> <u>Cutoff gait spd.</u>
≤ 173 cm ≥ 7 sec. ≤ 0.65 m/s
> 173 cm ≥ 6 sec. ≤ 0.76 m/s

Women

<u>Height</u> <u>Cutoff time</u> <u>Cutoff gait spd.</u>
≤ 159 cm ≥ 7 sec. ≤ 0.65 m/s
> 159 cm ≥ 6 sec. ≤ 0.76 m/s</td>
<td>Baseline height: F102, item 4

Follow up height: F304

Baseline gait speed (time needed to cover 4 m): F108, item 4

Follow up gait speed (time needed to cover 4 m): F308, item 4

We measure time it takes to cover 4 meters and Fried et. al have their criteria as the time it takes to cover 15 feet (4.572 m). In any case we can convert this criterion and our measurements into gait speed and use it this way (I calculated gait speeds on the left based on Fried's cutoffs).</td>
</tr>
<tr>
<td><u>Grip strength:</u>

Men

<u>BMI</u> <u>Cutoff grip strength (Kg)</u></td>
<td>We have baseline and follow up height and weight, so we have BMI.

Baseline grip strength: F108, item 10</td>
</tr>
</table>

≤ 24 ≤29 24.1 – 26 ≤ 30 26.1 – 28 ≤ 30 > 28 ≤ 32 **Women** BMI Cutoff grip strength (Kg) ≤ 23 ≤ 17 23.1 – 26 ≤ 17.3 26.1 – 29 ≤ 18 > 29 ≤ 21	Follow up grip strength: F308, item 10 We measure grip strength in kPa: 1kPa = 101.97 kgf/m^2
Frailty definition: Frail: ≥ 3 criteria present Pre-frail: 1 or 2 criteria present	

Level of physical activity. Physical activity is assessed by an *excerpt* from the Nurses' Health Study (NHS) questionnaire. The NHS physical activity questionnaire is well validated against incident chronic diseases. The endpoint is evaluated in all 2152 participants at baseline, 12, 24, and 36 months.

Commented [ES18]: The NHS physical activity questionnaire gives enough information to calculate METs. Do we use METs for this endpoint?

Commented [pchoca19R18]: I suggest we use METs.

Cardiovascular

Incidence of major cardiovascular eventsis assessed as a composite endpointevery 3 months (4 clinical visits and 9 phone calls) in all 2152 participants; the events are confirmed by medical records reviewed by and the Independent Physician Endpoint Committee based on pre-defined diagnostic cards.

- *Any event:* myocardial infarction, stroke, revascularization procedures of CABG and PCI, incident congestive heart disease (heart insufficiency), cardiovascular mortality.
- *Individual cardiovascular endpoints:* myocardial infarction, stroke, incident congestive heart disease and cardiovascular mortality

Brain

Incidence of dementia. Any new diagnosis of dementia (self-reported or proxy-reported)is confirmed by medical records and reviewed by the Independent Physician Endpoint Committee based on medical reports and on documented changes in the MoCA scores. The endpoint is evaluated at baseline, 12, 24, and 36 months in all 2152 trial participants.

Immunity

Incidence of canceris assessed as a composite endpointevery 3 months (4 clinical visits and 9 phone calls) in all 2152 participants; all cancer events are confirmed by medical records and reviewed by the Independent Physician Endpoint Committee based on pre-defined diagnostic cards.

- Any cancer: any confirmed diagnosis of malignant neoplasm
- Specific cancers: gastro-intestinal, breast cancer in women, prostate cancer in men.

Number of implant infections after total hip or knee replacement due to fracture or osteoarthritisisassessed every 3 months (4 clinical visits and 9 phone calls) in all 2152 trial participants. Each case of implant associated infection is confirmed by medical recordsand reviewed by the Independent Physician Endpoint Committee based on pre-defined diagnostic cards. This endpoint is defined as total number of implant infections after total hip or knee replacement due to fracture or osteoarthritis experienced by a participant during the follow up period.

Number of gastro-intestinal infectionsisassessed every 3 months (4 clinical visits and 9 phone calls) in all 2152 trial participants.Each case of gastro-intestinal infection is confirmed by medical records and reviewed by the Independent Physician Endpoint Committee based on pre-defined diagnostic cards.This endpoint is defined as total number of gastro-intestinal infections experienced by a participant during the follow up period.

Bone/Cartilage-Arthritis

Incidenceof symptomatic osteoarthritisis assessed in all 2152 trial participants at 12,24,and 36 months of follow-up and defined as a newly diagnosed case of osteoarthritis in a participant.

- **symptomatic knee osteoarthritis** will be assessed based on clinical examination (ACR criteria for knee OA (11)) and KOOS (12)
- **symptomatic hip osteoarthritis**is assessed based on clinical examination (ACR criteria for hip OA (21)) and theHip injury and Osteoarthritis Outcome Score**(**HOOS (22))
- **symptomatic hand osteoarthritis**is assessed with the *Quick*DASH outcome measurefor hand OA (23) supported by the assessment of painful joints.
- **Incident symptomatic knee, hip or hand osteoarthritis** is also evaluated as a composite endpoint. All incident diagnosed cases of knee, hip or hand OA will be recorded for this endpoint.For this outcome only the first diagnosis of OA in either knee, hip, or hand will be counted (i.e.: if a participant who has previously been diagnosed with knee OA also develops OA in the hand, this case will not be counted again).

Severity of hip pain in those with prevalent symptomatic hip OAis assessed at 12,24,and 36 months of follow-upby HOOS (22), a validated instrument for hip pain evaluation.Severity of hip pain is defined as the most severe pain category indicated by a participant in the Pain section of HOOS questionnaire (qq. P1 – P9).

Severity of hand pain in those with prevalent symptomatic handOAisassessed at 12,24,and 36 months of follow-upby the *Quick*DASHoutcome measure (23). This endpoint will be assessed by question 9 (hand section) of *Quick*DASH questionnaire and will be analyzed as a categorical variable.

3.2.5. Ancillary Fracture healing study (pending funding support by the AO Foundation)

This endpoint will assess fracture healing at 3 levels in individuals with incident major osteoporotic fractures at the arm (shoulder, humerus, forearm) and leg (hip, femur, ankle). All participants are encouraged to use the telephone hotline of their respective recruitment center to report an incident

fracture within 7 days (there will be a reminder at each 3 month in person contact). Three endpoints will be assessed for fracture healing:

Primary fracture healing endpoint

Rate of clinical fracture healingwillbe assessed with 3 additional phone calls at 6, 12, 18 weeks after the fracture using PROMIS-HAQ questionnaire as an assessment tool. The results will be compared to the baseline, 12, 24, or 36 monthPROMIS-HAQ measurements obtained for the same person as a part of DO-HEALTH endpoint evaluation (see Secondary Endpoints, Incident disability regarding activities of daily living).

Secondary fracture healing endpoint

Rate of functional fracture healingwill be measured with SPPB and grip strength test at regular 12, 24, and 36-month DO-HEALTH visits. Analysis for this endpoint will be adjusted for time elapsed since the fracture.

Exploratory fracture healing endpoint

Rate of radiological fracture healing will be assessed at an independent radiology institute using standard x-rays performed at the local hospitals for early (6 – 8 weeks) and late (12 – 14 weeks) fracture consolidation assessment. All x-rays will be de-personalized by the recruitment center and sent to the coordinating center at the University of Zurichfor independent assessment.

Commented [ES20]: Criteria for radiologic fracture healing heed to be defined.

3.3. Derived variables

Commented [pchoca21]: Maybe we should omit this for now

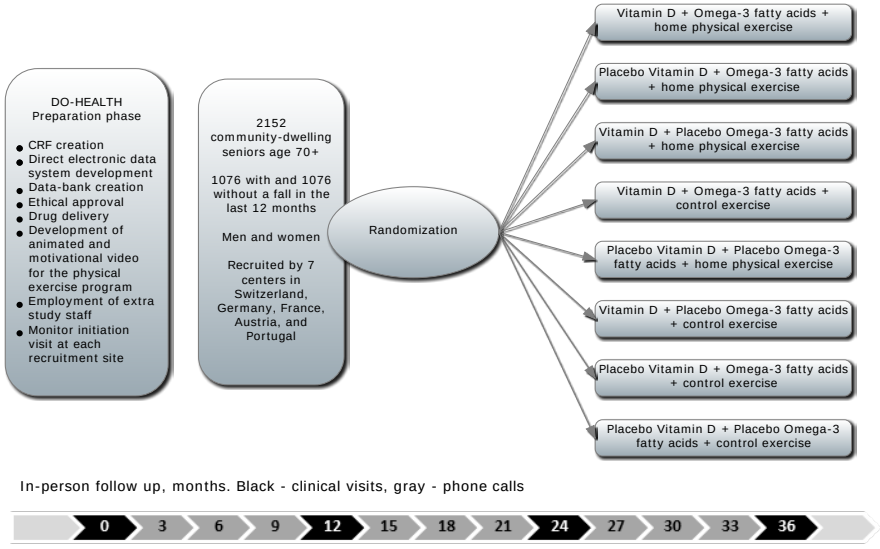
I will fill this in if needed after I have run the models on a mock data set. It is difficult to predict if we need to derive any variables from the data set otherwise.

4. Study Methods

4.1. General Study Design and Plan

DO-HEALTH is a randomized, double-blind, placebo-controlled, 2×2×2 factorial, multi-center clinical trial. The trial is performed at 8 recruitment centers located in 5 countries (Figure 1): Switzerland (University of Zurich, Basel University Hospital, Geneva University Hospital), France (University of Toulouse Hospital Centre), Germany (Charité Berlin, Friedrich-Alexander University Erlangen - Nurnberg), Portugal (University of Coimbra), and Austria (Innsbruck Medical University).

Figure 1. DO-HEALTH study schema



DO-HEALTH follow up consists of 4 clinical visits (baseline, 12, 24, and 36 months) and 9 phone calls every 3 months between the clinical visits (Figure 1).

4.2. Inclusion-Exclusion Criteria and General Study Population

DO-HEALTH clinical trial recruits a total of 2152 seniors aged 70 years and older living in the community; 50% of the trial population experienced a fall with or without a fracture within a year prior to enrollment into the study.

Inclusion criteria:

- Age ≥ 70 years
- Mini Mental State Examination Score of at least 24
- Living in the community
- Sufficiently mobile to reach the study center
- Able to walk 10 meters with or without a walking aid and getting in and out of a chair without help
- Able to swallow study capsules
- Able and willing to participate, sign informed consent (including consent to analyze all samples until drop-out or withdrawal) and cooperate with study procedures

Exclusion criteria:

- Consumption of more than 1000 IU vitamin D/day in the 6 months prior to enrollment, or a bolus of 300'000 IU or more in the last 12 month prior to enrollment, and unwillingness to reduce to 800 IU/d of vitamin D (current standard of care) for the duration of the trial
- Unwilling to limit calcium supplement dose to a maximum of 500 mg per day for the duration of the trial
- Taking omega-3 fat supplements and unwilling to forgo their use for the duration of the trial
- 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
- Current or recent (previous 4 months) participation in another clinical trial, or plans of such participation in the next 3 years
- 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
- Severe renal impairment (creatinine clearance ≤ 15 ml/min) or dialysis,
- Hypercalcaemia (> 2.6 mmol/l)
- Hemiplegia or other severe gait impairment
- History of hypo- or primary hyperparathyroidism
- History of granulomatous diseases (i.e. tubercolosis, sarcoidosis)
- Severe liver disease
- Major visual or hearing impairment or other serious illness that would preclude participation
- Living with a partner who is enrolled in DO-HEALTH (i.e. only one person per household can be enrolled)
- Living in assisted living situation or in nursing home
- Temporary exclusion: acute fracture in the last 6 weeks

4.3. Randomization and Blinding

General information

Participants are randomized to one of the eight treatment groups using stratified block randomization strategy (block size - 16 individuals). Four stratification variables are used: study center, fall during previous 12 months (yes/no), age (70 – 84 and 85+), and gender. Two stratification variables – study center and fall during previous 12 months (yes/no) – are strictly enforced: each study center must balance the recruitment of participants with (50%) and without a fall (50%) in the last year prior to enrollment. The other two stratification variables – age (70 – 84 and 85+) and gender – are monitored and corrective recruitment measures are taken if one of these strata contains less than 30% of the study participants. The randomization procedure is computerized; it is performed and monitored by DO-HEALTH randomization software.

The following procedures *must* have been completed*before randomization*:

1. Participant's Informed consent form is signed.

2. Serum calcium was measured and the measurement was below 2.6 mmol/L.
3. Serum creatinine was measured, creatinine clearance was calculated according to the Cockcroft-Gault formula(24), and was above 15 ml/min.
4. The study MD has seen the participant and confirmed all inclusion and exclusion criteria.
5. The mini mental state examination (MMSE) was applied with the participant and the participant reached a score > 24.
6. The participant is able to walk 10 meters with or without walking aid and is able to get in and out of a chair without help (simple screening test by a study nurse).

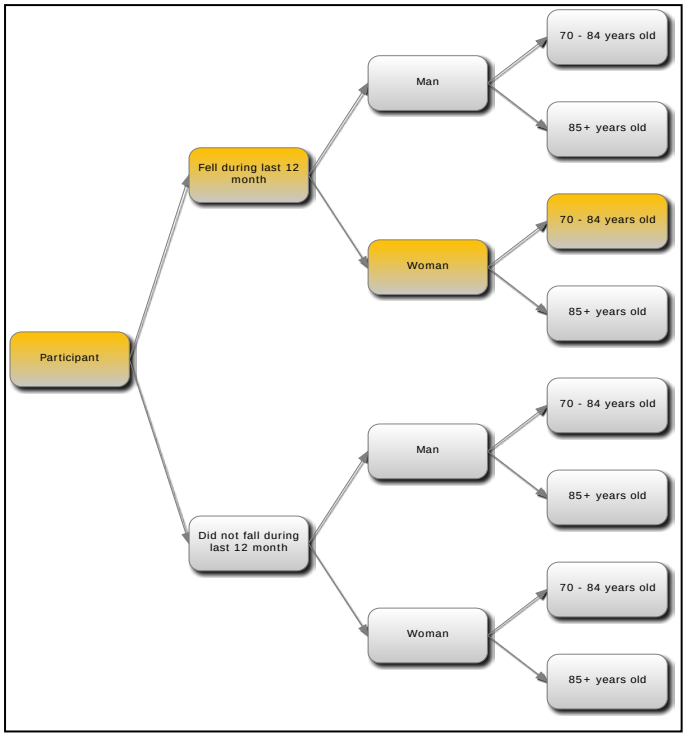
Not fulfilling any one of the conditions above disqualifies the participant from randomization.

Randomization procedure

Randomization is computerized. Each center has to access the randomization software with its unique login and provide information on the stratification variables for the new participant. Based on this information, the software issues the subject ID and intervention/exercise package code. Stratification flowchart is presented in Figure 2; highlighted rectangles represent randomization strata for a 72 year-old woman who fell during last year. Each randomization center applies a separate but identical structure to identify the strata for randomization.In case the recruitment center has an internet access problem, the coordinating center at the University of Zurich is contacted and performs randomization for that center.

Upon submitting information on prior fall, gender, and age to identify the strata, the DO-HEALTH randomization software provides the subject ID and intervention/exercise package code. Each of the recruitment centers has a separate pool of subject IDs and package codes. The package codes provided by the software correspond to a pre-coded supply of study intervention (12 bottles per code per year) and a supply of exercise material (video + written manual + rubber bands) stored at that randomization center. The participant is considered randomized once the subject ID has been issued and assigned to him/her. The intervention package code is used to match the study interventionto the subject ID after randomization. Each bottle of the study interventionislabeled with the ID in addition to the intervention code. At each visit the participant receives 12 bottles of the study intervention to provide one-year supply.

Figure 2. Determining randomization strata.



Blinding

The computerized randomization procedure ensures that both study participants and study personnel are blinded with regards to treatment allocation. The randomization software can perform un-blinding procedure and reveal which treatment has been assigned to a particular participant if un-blinding has been deemed necessary for safety of the participant or regulatory purposes. The un-blinding procedure can be performed only by the Principal Investigator (PI) or study personnel with the highest clearance to the randomization software.

4.4. Study Variables

Table 1. Study variables and periodicityof assessment

Variables	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Primary Endpoints													
Incident non-vertebral fractures	X	X	X	X	X	X	X	X	X	X	X	X	X
SPPB score	X				X				X				X
Blood pressure	X				X				X				X
MoCA score	X				X				X				X
Incident infection	X	X	X	X	X	X	X	X	X	X	X	X	X
Secondary Endpoints													
Incident hip fractures	X	X	X	X	X	X	X	X	X	X	X	X	X
Incident vertebral fractures*	X				X				X				X
Incident fracture of any type*	X	X	X	X	X	X	X	X	X	X	X	X	X
BMD at the spine and hip*	X				X				X				X
Incident low trauma falls	X	X	X	X	X	X	X	X	X	X	X	X	X
Incident injurious fall	X	X	X	X	X	X	X	X	X	X	X	X	X
Number of participant who fell	X	X	X	X	X	X	X	X	X	X	X	X	X
Reaction time	X				X				X				X
Grip strength	X				X				X				X
Muscle mass in the upper and lower extremities*	X				X				X				X
Musculoskeletal pain	X				X				X				X
Dual tasking gait speed	X				X				X				X
Incident hypertension	X				X				X				X
Mental health decline	X				X				X				X
Incident depression	X				X				X				X
Dual tasking gait variability†	X				X				X				X
Incident upper respiratory infection	X	X	X	X	X	X	X	X	X	X	X	X	X
Incident flu-like illness	X	X	X	X	X	X	X	X	X	X	X	X	X

Variables	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Incident infection that led to hospitalization	X	X	X	X	X	X	X	X	X	X	X	X	X
Prevalent symptomatic OA	X												
Knee pain	X				X				X				X
Knee buckling	X				X				X				X
Number of painful joints	X				X				X				X
NSAID use due to knee pain	X				X				X				X
Oral health (GOHAI)	X				X				X				X
Tooth loss	X	X	X	X	X	X	X	X	X	X	X	X	X
Prevalent gastro-intestinal symptoms	X				X				X				X
Fasting blood glucose	X				X				X				X
Fasting blood insulin	X				X				X				X
Body composition*	X				X				X				X
Body fat mass*	X				X				X				X
Serum creatinine	X				X				X				X
Glomerular filtration rate	X				X				X				X
Quality of life	X		X		X		X		X		X		X
Frailty	X				X				X				X
Disability regarding activities of daily living	X				X				X				X
Nursing home admission	X	X	X	X	X	X	X	X	X	X	X	X	X
Hospitalizations	X	X	X	X	X	X	X	X	X	X	X	X	X
All-cause mortality	X	X	X	X	X	X	X	X	X	X	X	X	X
Biomarker Endpoints													
Serum calcium	X				X				X				X
Serum phosphate	X				X				X				X
Serum 25(OH)D	X				X				X				X
Intact PTH	X				X				X				X

Variables	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Urinary calcium/creatinine ratio (second spot urine)	X				X				X				X
Beta-Crosslaps serum	X				X				X				X
P1NP	X				X				X				X
Sclerostin	X				X				X				X
Myostatin	X				X				X				X
Troponin T	X				X				X				X
NT-proBNP	X				X				X				X
homocysteine	X				X				X				X
Creatin kinase	X				X				X				X
LDL	X				X				X				X
Cholesterol	X				X				X				X
HDL	X				X				X				X
Triglycerides	X				X				X				X
CRP	X				X				X				X
high sensitivity-CRP	X				X				X				X
Interleukin 6	X				X				X				X
ALT	X				X				X				X
AST	X				X				X				X
Gamma Glutamyl Transpeptidase	X				X				X				X
Alkaline phosphatase	X				X				X				X
Bilirubin	X				X				X				X
Serum creatinine	X				X				X				X
Serum urea	X				X				X				X
Uric acid	X				X				X				X
Sodium	X				X				X				X
Potassium, chloride, magnesium	X				X				X				X
Chloride	X				X				X				X
Magnesium	X				X				X				X

Variables	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Total protein	X				X				X				X
Albumin	X				X				X				X
Ferritin	X				X				X				X
Transferrin	X				X				X				X
Thyroid stimulating hormone (TSH)	X				X				X				X
Free Thyroxin (fT4)	X				X				X				X
Free Triiodothyronin(fT3)	X				X				X				X
Cortisol	X				X				X				X
Folic acid	X				X				X				X
Vitamin B12	X				X				X				X
Plasma Eicosapentaenoic Acid (EPA)	X				X				X				X
Plasma Arachidonic Acid (AA)	X				X				X				X
Plasma Docosahexaenoic Acid (DHA)	X				X				X				X
Tumor necrosis factor α (TNF-α)‡	X				X				X				X
Interleukin 10‡	X				X				X				X
Interleukin 17‡	X				X				X				X
Interleukin 22‡	X				X				X				X
~~Number of CD16/56 positive natural killer cells~~CD3‡	X				X				X				X
~~Number of regulatory T cells~~CD4‡	X				X				X				X
CD25‡	X				X				X				X
CD127‡	X				X				X				X
Exploratory Endpoints													
Physical Activity	X				X				X				X
Incident major cardiovascular evens	X	X	X	X	X	X	X	X	X	X	X	X	X
Incident dementia	X				X				X				X
Incident cancer	X	X	X	X	X	X	X	X	X	X	X	X	X

Variables	Baseline visit	Phone Call 1 3 M	Phone Call 2 6 M	Phone Call 3 9 M	Visit 1 12 M	Phone Call 4 15 M	Phone Call 5 18 M	Phone Call 6 21 M	Visit 2 24 M	Phone Call 7 27 M	Phone Call 8 30 M	Phone Call 9 33 M	Visit 3 36 M
Incident implant infections after total hip or knee replacement due to fracture or osteoarthritis	X	X	X	X	X	X	X	X	X	X	X	X	X
Incident gastrointestinal infection	X	X	X	X	X	X	X	X	X	X	X	X	X
Incident symptomatic OA (knee, hip, or hand)					X				X				X
Severity of hip pain					X				X				X
Severity of hand pain					X				X				X

*Variable is assessed in 1502 participants at 5 recruiting centers equipped with iDXA machines.
† Variable is assessed in 250 participants recruited at Basel University.
‡ Variable measured in ~~700~~ 802 participants recruited in Basel and~~,~~ Zurich~~, and Geneva~~.

5. Sample Size

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

Power estimates for the primary endpoints are summarized in Table 2. Please refer to the DO-HEALTH study protocol for detailed discussion and power calculations.

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

Endpoint	Minimum detectable difference[†]	Standard Deviation	Alpha	Power with no interact., N = 1806 (903 + 903)	Power with interaction, N = 902 (451 + 451)
Bone:[‡]					
Incidence rate of any non-vertebral fracture including hip fractures (proportion)	0.073[¶]	–	0.01	0.99	0.80
Muscle:[**]					
lower extremity function (mean SPPB score)	0.40	1.41	0.01	0.99	0.97
Cardiovascular:[**]					
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
Brain:[**]					
Cognitive function (mean MMSE score)	0.7	2.3	0.01	0.99	0.90
Immunity:[††]					
Incidence rate of any infection	0.32	$s_1 = 1.46$ $s_2 = 1.35$	0.01	0.99	0.90

[*] All assumptions about means and standard deviations of the parameters are based on the results of pilot trials to DO-HEALTH(9, 25, 26) and research of other authors(27-29).
[†] Minimum detectable difference was assumed as a minimum difference that is clinically significant.
[‡] Power calculations are based on the Z-test for difference between two proportions.
[¶] Estimated baseline prevalence of any non-vertebral fractures incidence rate / IR = 0.14.
[**] Power calculations are based on the two-sample t-test assuming equal variances.
[††] Power calculations are based on Poisson regression assuming non-equal variances.

6. General Considerations

6.1. Timing of Analyses

The final analysis will be performed after all 2152 participants have completed their 36-month clinical visit or dropped out of the trial. Prior to the analysis a status of each study participant should be ascertained as either "completed 36 months of follow up" or "dropped out". All data must be transferred from the study centers and stored at the Central Databank. All blood samples must be analyzed for biomarkers, the data transferred to the Central Databank, and linked to the data base before the biomarker analysis should commence.

Analyses limited to the baseline data may occur at any time after the final patient has been randomized.

6.2. Analysis Populations

6.2.1. Primary Analysis Population

All primary analysis in DO-HEALTH will be conducted according to the intention to treat (ITT) principle. Every study participant who was randomized and receivedtheDO-HEALTH treatment kit at baseline clinical visit will be a part of the primary analysis population.

6.2.2. Per Protocol Population

In addition to the primary ITT analyses, the same data will be analyzed in participants who demonstrated acceptable adherence to the study protocol. The per protocol analyses will be treated as sensitivity analyses. The per protocol analysis population will include the participants who:

- Attended at least 2 out of 3 follow up clinical visits;
- Participated in at least 7 out of 9 follow up phone calls;
- Took at least 80% of the study medication as confirmed by pill count;
- Did not take additional vitamin D or calcium supplements in doses exceeding those stipulated by the Exclusion Criteria (see Section 4.2 for details), or take any additional omega-3 supplements or vitamin Dmetabolites.
- Reported doing the DO-HEALTH exercise program at least once a week (on average) for the duration of the trial.

6.2.3. Safety Population

Safety analyses will be performed on the safety population, which will be comprisedof all randomized subjects who have received the DO-HEALTH treatment kit at the baseline visit.The safety population is equivalent to the primary analysis population (see Section 6.2.1).

6.2.4. Special Study Populations

Several endpoints will be measured in a priori defined subsets of the study population. Apart from the total sample size, all definitions for the primary analysis population (Section 6.2.1) and the per protocol

population (Section 6.2.2) fully apply to these sub-populations. The endpoints and corresponding subpopulations are summarized in Table 3.

Table 3. Special sub-populations used to measure DO-HEALTH endpoints

Endpoints	Sub-population description	Sample size
Incident vertebral fractures Incident fracture of any type BMD at the spine and hip Muscle mass in the upper and lower extremities Body composition Body fat mass	Participants at 5 recruiting centers equipped with iDXA machines (Zurich, Berlin, Toulouse, Nurnberg, and Coimbra)	1502
Dual tasking gait variability	Participants recruited at Basel University	250
Tumor necrosis factor α (TNF-α) Interleukin 10 Interleukin 17 Interleukin 22 ~~Number of CD16/56-positive natural killer cells~~CD3 CD4 CD25 ~~Number of regulatory T cells~~CD127	Participants recruited in Basel and~~,~~ Zurich~~, and Geneva~~	~~700~~802

6.3. Covariates and Subgroups

In addition to the primary analyses of main effects of vitamin D_3 and/or omega-3 supplementation and/or simple home exercise program, a series of analysis adjusting for *a priori* determined covariates will be performed. The aims of the analysis is to determine whether the separate and combined effects of vitamin D_3 and/or omega-3 supplementation and/or simple home exercise program on the primary and secondary endpoints are different among pre-determined subgroups or significantly changed by the covariates.

The following covariates will be considered:

- Age at baseline
- Gender
- Body Mass Index (BMI);
- Baseline physical activity;
- Baseline serum 25(OH)D levels;
- Baseline serum PUFA levels;
- Baseline calcium intake;
- Baseline protein intake;
- FRAX®-estimated absolute fracture risk.

The analyses will consider the following pre-defined subgroups:

- Gender (men; women);
- Age (70 – 84; 85+ years);
- A fall in during the 12 months preceding recruitment (yes/no);
- A fracture during 10 years preceding recruitment (yes/no);
- Baseline symptomatic knee OA (yes/no).

6.4. Missing Data

Computer-based data collection during clinical visits and follow-up phone calls along with software-based data quality control procedures should greatly reduce the frequency of missing data. However, missing data can still occur. The following sections summarize the strategies that we intend to use dealing with missing data.

6.4.1. Missing primary outcomes data

Missing data on any of the 5 primary outcomes will be assumed to be missing at random (MAR) meaning that the events that caused the missing data do not depend on the missing data themselves.Thus,the data from the study population will be assumed to provide unbiased estimates of treatment effect and missing data will be 'ignored' in the primary analysis(30).However if greater than 1% of data on each primary outcome that should be available for analysis are missing, a sensitivity analysis for that primary outcome will be undertaken in addition to the primary MAR analysis.

In the sensitivity analysis the missing data will be imputed by: 1) last observation carried forward; 2) worst extreme case imputation and; 3) regression model imputation using all available information(31). Results of the primary MAR analysis will be interpreted in the context of these sensitivity analyses.

Information that is *unavailable* for analysis due to withdrawal of consent for data use will not be considered *missing* and therefore will not be included in the estimate of percent missing as described above nor will it be included in the sensitivity analysis.

6.4.2. Missing data on secondary, biomarker, and exploratory outcomes

The assumptions and strategies used to dealing with missing data on secondary, biomarker, and exploratory endpoints will be the same as for primary endpoints (see Section 6.4.1), but the minimum percentage of missing data to warrant a sensitivity analysis will be 5%.

6.4.3. Missing covariates data

Exclusion of randomized patients with known outcomes from analysis, for any reason, contravenes the intention to treat principle. Every effort will be made to minimize post-randomization exclusions.

SAS statistical software package requires complete information on all covariates for a patient case to be included in a covariate adjusted regression model. Any missing informationfrom any covariate results in

the exclusion of the entire patient case. Exclusion of incomplete cases with known outcomes reduces statistical power and can introduce bias into the treatment effects estimates.

Missing baseline covariate will be replaced with mean values calculated from the observed non-missing instances of that baseline covariate within the same study center and randomization stratum (fracture history / age / gender) to which the patient case with missing data belongs. The imputed means will be calculated using pooled data from all treatment arms.

If a covariate value from a follow up visit or phone call is missing, this value will be imputed via one of the following strategies: (1) if missing values occurred due to death or dropout of the participant (endpoint information is not ascertained for this and all subsequent calls/visits) , no imputation will be done; (2) if the participant did not drop out, but no information on a particular covariate exists beyond this visit, the last known value for the covariate will be carried forward until the follow-up is complete or the participant dropped out; (3) if participant data include information for time points before and after the time point for which the covariate data is missing, the missing data will be imputed with the mean calculated from the values obtained for this participant at time points before and after the one for which the data are missing.

Replacement values for missing calculated constructs such as BMI and SPPB score will be estimated using non-missing component-level information whenever possible. For example, if one of the components of BMI is missing, such as height, overall randomization stratum specific mean height will be imputed and BMI will be calculated with the known weight and imputed mean height.

If a baseline prognostic variable requires imputation of missing values, the percent of cases that were originally missing will be reported. In addition, for sensitivity analysis the data will be re-analyzed with all participant cases with missing data excluded from the analysis. Results of the primary analysis will be interpreted in the context of this sensitivity analysis.

6.5. Data Monitoring and Interim Analyses

Progress toward recruitment goals and balance within the recruitment strata in all study centers will be constantlymonitored by the DO-HEALTH randomization software. The software will issue warnings if a center falls too far behind its recruitment goals or if a significant imbalance among the randomization strata is detected (refer to section 10 “Data Management” of the DO-HEALTH Study Protocol for detailed description). Safety and adherence data will be collected from all study participants every 3 or 12 months as appropriate. Please refer to sections 7.4, 8.6.2, and 12.3 for detailed description of study safety and adherence endpoints assessment.

No interim analysis is foreseen. All recruitment, safety, and adherence data will be presented to the Data Safety and Monitoring Board (DSMB) at periodic meetings. An interim analysis may be performed only if the DSMB, upon reviewing the recruitment and safety data, deems them necessary and explicitly requests the analysis to be performed. In this case, the interim analysis will be performed only to the extent that satisfies the DSMBrequest.

6.6. Multiple Testing

The trial has five primary outcomes: the rate of any non-vertebral fractures (rate comparison), the Short Physical Performance Battery (continuous), blood pressure (continuous), cognitive decline (continuous), number of infections (Poisson count). Bonferroni adjustment will be used to account for multiple comparisons so that $p < 0.01$ is required for statistical significance.

7. Summary of Study Data

All continuous variables will be summarized using the following descriptive statistics: N (non-missing sample size), mean, standard deviation, median, minimum, and maximum. For all categorical variables frequencies and percentages (based on the non-missing sample size) of observed levels will be reported. All study data will be presented sorted by study center, treatment, and visit; a summary combining all study centers will be presented sorted by treatment and visit. All summary tables will be structured with a column for each treatment in the following order: Placebo, Vitamin D supplement only, Omega-3 fatty acids supplement only, Muscle strength exercise only, Vitamin D + Omega-3 fatty acids supplements, Vitamin D supplement + Muscle strength exercise, Omega-3 fatty acids supplement + Muscle strength exercise, Vitamin D + Omega-3 fatty acids supplements + Muscle strength exercise. All tables will be annotated with the total population size relevant to that table/treatment, including any missing observations.

7.1. Participant Disposition

Participant disposition throughout the trial will be established by the following conditions:

1. Screened: any senior who signed the informed consent and attended the screening session at baseline clinical visit.
    a. Software ID is assigned to the person,
    b. The person is enrolled into the study via the DO-HEALTH software (the software does not allow enrollment unless the "informed consent signed" flag is checked, the date of the signature is recorded, and the enrollment is confirmed by a personal password of a DO-HEALTH clinical staff member).
2. Eligible: ascreened seniorwho satisfied all inclusion/exclusion criteria at baseline and has been deemed eligible for the trial regardless of whether this person was randomized or not (the person is "screened in" for the baseline visit by the DO-HEALTH software).
3. Randomized: an eligible senior who was randomized for a treatment group and assigned a randomization number by the DO-HEALTH software.
4. Completed baseline visit: a randomized study participant who completed baseline assessments.
    a. All F1xx forms are on file, all participant-reported questionnaires are checked out as "complete" by the DO-HEALTH software, "End of visit checklist (Baseline)" from is complete, signed and present in the participants folder.

5. Completed a follow up phone call: a study participant who has completed a 3-monthly follow up phone call evaluation.
    a. F201 is on file for a 3-monthly phone call.
6. Completed 12, 24, and 36 month clinical visit: a study participant who completed yearly assessments at 12, 24, and 36 months of follow up.
    a. All F3xx forms are on file, all participant-reported questionnaires are checked out as "complete" by the DO-HEALTH software for each clinical visit, "End of visit checklist (12, 24, or 36 months)" from is complete, signed and present in the participants folder.
7. Dropout: a randomized participant is considered a dropout if they (1) notified the study personnel of their intention to discontinue their participation, (2) withdrew their informed consent, or (3) have not participated in follow up phone calls / clinical visits for more than 6 months and could not be contacted despite the reasonable effort to do so.
    a. Dropout form is filled in, signed, and filed in the participants folder

Participant disposition is summarized in Figure 3.

Commented [ES22]: These will need to be amended once all data collection tools are electronic. I have defined these disposition in terms of the mixture of paper/electronic forms we have now. Ideally we will identify a set of key variables in the data set that will define each disposition. A combination of visit date and "End of the visit checklist" could be a good candidate for clinical visits and contact date + completed F201 – for the phone calls.

Figure 3. Participant disposition flowchart

7.2. Demographic and Baseline Variables

The following demographic variables and potential confounders are measured at baseline:

1. Age
2. Sex
3. History of fractures 10 years prior to enrollment
4. FRAX score
5. Comorbidity (Sangha's Index)
6. Dietary history
7. Sunlight exposure.

7.3. Concurrent Illnesses and Medical Conditions

Detailed information on concurrent illnesses and medical conditions will be obtained at baseline 12, 24, and 36 month clinical visits. This information will be available in the DO-HEALTH data base both as individual variables indicating presence or absence of a certain medical condition and as Sangha's comorbidity index.

7.4. Treatment Compliance

Compliance to the study medication will be assessed at follow-up phone calls and clinical visits. Every 3 months during a follow up phone call each participant is asked whether he or she takes their medication and does the physical exercise program regularly. Participantswho have been takingtheir medication at least 6 days a week are considered to be compliant to the study medication regimen. Participants that do their physical exercise at least 1 time a week are considered compliant with respect to the physical activity component. Compliance information will be stored at the data base as two variablesfor each follow-up phone call: medication compliance (Yes/No) and physical exercise compliance (Yes/No).

Commented [ES23]: These are my definitions of compliance. They need to be confirmed by Heike. The definitions are roughly 80% of study medication or physical exercise. Currently compliance information is recorded by a questionnaire. The questionnaire is quite detailed, but I would advise to add two Yes/No questions asking whether a participant is compliant with the medication regimen and the physical exercise program.

Compliance will be further verified at follow up clinical visits at 12, 24, and 26 months.Remaining capsules will be counted and the information will be stored in the data base as two variables: the actual number of remaining capsules and the percentage out of 744 capsules – the 1-year supply of study medication capsules that each participant receives. Patients that took at least 80% of the study medication will be considered compliant to the study medication regimen. Compliance to the exercise pro will be assessed by the questionnaire in the same was as described above for the follow-up phone calls. Compliance information will be stored at the data base as two variablesfor each follow-up phone call: medication compliance (Yes/No) and physical exercise compliance (Yes/No).

8. Efficacy Analyses

8.1. Primary Efficacy Analysis

All assessment of treatment effects in the primary efficacy analysis are based on the intent-to-treat principle. All endpoint variables will be summarized by treatment group. N, Mean, Standard Deviation, Minimum and Maximum will summarize continuous endpoint variables and Number and Percentage will summarize categorical endpoint variables.

To account for multiple testing, all primary endpoints will be tested at the 2-sided 1% significance level (α = 0.01); all other endpoints will be tested at 5% significance level (α = 0.05, 2-sided).

8.1.1. Primary Endpoints

Effects of the study treatments on **incidence rate of non-vertebral fractures**and **incidence rate of any infection** will be evaluated by Poisson regression models:

$$Log(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Fall + \gamma_1(VD \times \Omega 3) + \gamma_2(VD \times PE) + \gamma_3(\Omega 3 \times PE) + \\ + \gamma_4(VD \times \Omega 3 \times PE) + log(Time) + \varepsilon \quad (1)$$

Commented [ES24]: If this endpoint turns out to be the risk of at least one fall, this models will have to be converted into a logistic model

Where:

Y – Primary endpoint (number of incident non-vertebral fractures or number of incident infections);
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo);
PE – physical exercise program (strength vs. mobility);
Fall –history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Time – follow up time.

Effects of the study treatments on**functional decline, change in systolic and diastolic blood pressure,** and **cognitive decline** will be evaluated by linear mixed models:

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Time_{ij} + \beta_5 Fall_i + \beta_6 Y\_BL_i + \gamma_1(VD_i \times \Omega 3_i) + \gamma_2(VD_i \times \\ PE_i) + \gamma_3(\Omega 3_i \times PE_i) + \gamma_4(VD_i \times \Omega 3_i \times PE_i) + \gamma_5(Time_{ij} \times VD_i \times \Omega 3_i) + \gamma_6(Time_{ij} \times VD_i \times PE_i) + \quad (2) \\ \gamma_7(Time_{ij} \times \Omega 3_i \times PE_i) + \gamma_8(Time_{ij} \times VD_i \times \Omega 3_i \times PE_i) + b_{1i} + b_{2i} Time_{ij} + \varepsilon_{ij}$$

Where:

i – i-th patient (i = 1, 2, …, 2152);
j – j-th visit (j = 1, 2, 3);
Y_{ij} – Primary endpoint (SPPB score, systolic and diastolic blood pressure, or MoCA score) for i-th patient at j-th visit;
$Time_{ij}$ – Follow up time since baseline for i-th participant at j-th visit; The exact formulation of the time variable (one first-order term, higher-order terms, spline model, or a categorical variable) will be determined for each outcome individually based on shapes of theobserved trends over time.

VD_i – vitamin D treatment (2000 IU/day vs. placebo) for the i-th participant;
$\Omega 3_i$ – omega-3 fatty acids treatment (1 g/day vs. placebo) for the i-th participant;
PE_i – physical exercise program (strength vs. mobility) for the i-th participant;
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Y_BL_i – baseline value of primary endpoint measurement (SPPB score, systolic and diastolic blood pressure, or MoCA score);

8.1.2. Secondary Endpoints

The following secondary endpoints will be analyzed with Poisson model (1) formulated above:

- Incidence rate of hip fractures
- Incidence rate of vertebral fractures
- Incidence rate of any fractures
- Incidence rate of any low trauma falls
- Incidence rates of injurious falls
- Incidence of depression
- Incidence rate of any upper respiratory infection
- Incidence rate of flu-like illness
- Incidence rate of severe infections that lead to hospital admission
- Incident disability regarding activities of daily living
- Rate of acute hospital admissions.
- Total number of painful joints at each time point

Linear mixed models formulated above (2) will be used for the following secondary endpoints:

- Bone mineral density (BMD) at the spine and hip
- Reaction time
- Grip strength
- Muscle mass in the upper and lower extremities
- Dual tasking gait speed
- Dual tasking gait variability
- Severity of knee pain
- Decline in kidney function (creatinine level and glomerular filtration rate)
- Fasting blood concentration of glucose
- Fasting blood concentration of insulin
- Body composition and fat mass
- General Oral Health Assessment Index
- Level of activities of daily living (HAQ score)
- Quality of life

Mathematical transformations of the endpoint measurements may be necessary if their distributions strongly deviate from normality.

Binary secondary endpoints.

For the following binary endpoints cumulative 36-month risk will be assessed:

- Risk of incident hypertension
- Incidence of depression
- Incident frailty
- Incident nursing home admissions
- Incident disability regarding activities of daily living
- Mortality.

These outcomes will be evaluated by two sets of models. The 36-month risk of each of the outcomes will be evaluated using the logistic model defined as follows:

$$Logit(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Fall + \gamma_1(VD \times \Omega 3) + \gamma_2(VD \times PE) + \gamma_3(\Omega 3 \times PE) + \gamma_4(VD \times \Omega 3 \times PE) + \varepsilon \quad (3)$$

Where all the variables are defined the same way as for the model (1) above.

Since follow-up times for the participants are likely to be different, we will alsocompare time until the outcomeamong the treatment groups using Cox survival models. The proportional hazard assumption will be checked for each outcome and, if the assumption is satisfied, the Proportional Hazards Cox model will be used; the Stratified Cox model will be used otherwise. Both types of the Cox model will be defined in the same way:

$$h(t, \mathbf{X}) = h_0 t \times exp[\beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Fall + \gamma_1(VD \times \Omega 3) + \gamma_2(VD \times PE) + \gamma_3(\Omega 3 \times PE) + \gamma_4(VD \times \Omega 3 \times PE)] \quad (4)$$

Where all the variables are defined the same way as for the model (1) above.

NSAID use due to knee pain

NSAIDuse due to knee pain will be evaluated at each clinical visit and the NSAID use status can potentially change from one visit to the next. This endpoint will be analyzed with the marginal logistic (GEE) model defined below:

$$Logit(Y_{ij}) = \alpha + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Fall_i + \beta_5 Time_{ij} + \beta_6 Y\_BL_i + \gamma_1(VD_i \times \Omega 3_i) + \gamma_2(VD_i \times PE_i) + \gamma_3(\Omega 3_i \times PE_i) + \gamma_4(VD_i \times \Omega 3_i \times PE_i) + \gamma_5(Time_{ij} \times VD_i \times \Omega 3_i) + \gamma_6(Time_{ij} \times VD_i \times PE_i) + \gamma_7(Time_{ij} \times \Omega 3_i \times PE_i) + \gamma_8(Time_{ij} \times VD_i \times \Omega 3_i \times PE_i), \quad (5)$$

Where:

Y_{ij} is a binary NSAID use variable (yes/no) which denotes NSAIDS use due to knee pain by the i-th study participant at j-th month and all the variables are defined the same way as for the model (2). Model parameters will be estimated using Generalized Estimating Equations. Model fit will be assessed by comparing a plot of observed cumulative sum of residuals against time to the simulated plots of the residuals coming from the appropriate normal distribution.

Prevalence of gastro-intestinal symptoms

Prevalence of gastro intestinal symptoms will be measured by Rome III questionnaire. The questionnaire will be scored according to the official SAS scoring procedure published by the Rome Foundation on their web site (http://www.romecriteria.org/rome_iii_sas/).

This endpoint will be analyzed both for presence/absence of each particular GI symptom at each clinical visit and for the total number of GI symptoms present at each clinical visit. For analyzes of the collection of *binary variables* (GI symptom present yes/no) the marginal logistic (GEE) model formulated identically to the model (5) above will be used. For this model Y will be a binary variable denoting presence or absence of each particular GI symptom diagnosed by the Rome III questionnaire. Count of GI symptoms diagnosed by the Rome III questionnaire at each clinical visit will be evaluated by the following marginal Poisson (GEE) model:

$$Log(Y_{ij}) = \alpha + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Fall_i + \beta_5 Time_{ij} + \beta_6 Y\_BL_i + \gamma_1(VD_i \times \Omega 3_i) + \gamma_2(VD_i \times PE_i) + \gamma_3(\Omega 3_i \times PE_i) + \gamma_4(VD_i \times \Omega 3_i \times PE_i) + \gamma_5(Time_{ij} \times VD_i \times \Omega 3_i) + \gamma_6(Time_{ij} \times VD_i \times PE_i) + \gamma_7(Time_{ij} \times \Omega 3_i \times PE_i) + \gamma_8(Time_{ij} \times VD_i \times \Omega 3_i \times PE_i), \quad (5a)$$

where Y_{ij} is a count of GI symptoms diagnosed by the Rome III in the i-th participant at j-th month and all the variables are defined the same way as for the model (2). Model fit assessment will be done as described for model (5) above.

Frailty

Frailty scores will be calculated using gender-specific DFactor score models proposed by Romero-Ortuno et al. (17).

For women: DFS (females) = (2.077707 × Fatigue − 0.757295) × 0.4088 + (3.341539 × Loss of appetite − 0.332289) × 0.3325 + (0.132827 × Grip strength − 3.534515) × (−0.4910) + (2.627085 × Functional difficulties − 0.461808) × 0.6012 + (0.918866 × Physical activity −1.523633) × 0.4818;

For men: DFS (males) = (2.280336 × Fatigue − 0.592393) × 0.3762 + (4.058274 × Loss of appetite−0.263501) × 0.3130+(0.092326×Gripstrength−3.986646) × 0.4653 + (3.098226 × Functional difficulties − 0.365971) × 0.6146 + (1.005942 × Physical activity −1.571803) × 0.4680.

Frailty will be defined as follows:

Women:

- If predicted DFS < 0.3151361243, NON-FRAIL
- If predicted DFS < 2.1301121973, PRE-FRAIL
- If predicted DFS < 6, FRAIL

Men:

- If predicted DFS < 1.211878526, NON-FRAIL
- If predicted DFS < 3.0052612772, PRE-FRAIL
- If predicted DFS < 7, FRAIL.

Proportions of frail seniors (frail vs. non-frail or pre-frail) among the treatment groups will be compared using a GEE model formulated identically to the model (5) above.

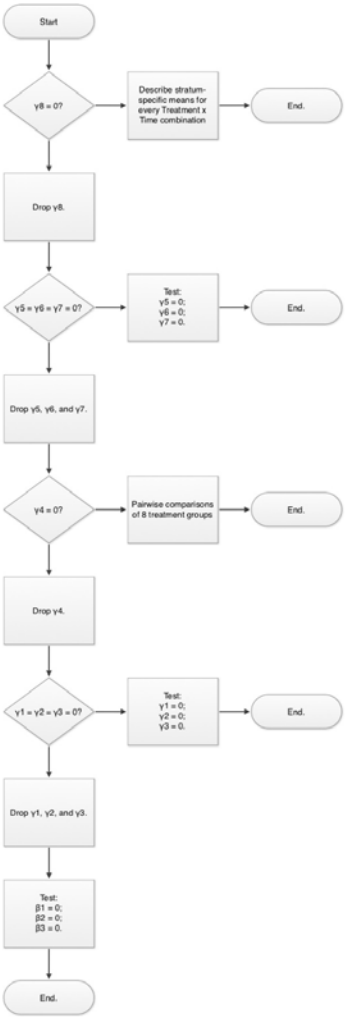
Categorical secondary endpoints.

Knee buckling is assessed by question 3 of the Knee Buckling Questionnaire. The question has 5 categories of answers evaluating the number of time participant's knees buckled during the past 3 months. To analyze this endpoint an ordinal logistic regression will be built for 12-, 24-, and 36-month time point. The proportional hazards assumption will be checked for each model, if the assumption does not hold, the model will be re-formulated as a multinomial logistic regression models.

Hypotheses testing strategy for all models

The modeling strategy for all models is depicted in Figure 4. The models that do not have as many parameters as shown on the flowchart will be built and tested following the same approach for the parameters available in the models. Interaction terms in the full models will be tested for statistical significance at $\alpha = 0.1$ using hierarchical backwards elimination procedure (32) starting with the highest orderterms. Interaction terms that are not statistically significant will be dropped from the model. To limit the number of statistical testing, interaction terms will be tested in chunks whenever possible and individual interaction terms will be tested only if a chunk-test is statistically significant. All first order terms will remain in the model regardless of their statistical significance.

Figure 4. Modeling strategy



8.2. Subgroup Analyses

In the subgroup analysis we will test whether vitamin D_3 and/or omega-3 fatty acids (EPA+DHA) and /or the simple home exercise program reduce the risk of primary and secondary endpoints differentially by gender, age (70-84; 85+), body mass index, baseline physical activity, baseline serum 25(OH)D levels, baseline PUFA levels, previous fall (last year), previous fracture (last 10 years), FRAX-estimated absolute fracture risk, baseline symptomatic knee OA, and baseline calcium and protein intake (diet + supplements).

The following subgroups will be considered for analyzes:

- Gender (men / women);
- Age (70 – 84 / 85+);
- Body mass index (≤18 / 18.1 – 25 / 25.1 – 30 / >30);
- Baseline physical activity (light / moderate / vigorous);
- Baseline serum 25(OH)D level,nmol/L (≤25 / 25.1 – 50 / 50.1 – 75 / >75);
- Baseline PUFA levels (quartiles)
- Fall during a year prior to recruitment (Yes / No);
- FRAX-estimated absolute 10-year fracture risk (<10% / 10 – 25% / 25 – 50% / >50%);
- Baseline symptomatic knee OA(Yes / No);
- Baseline calcium intake, mg/day (≤1200 / 1200+);
- Baseline protein intake (quartiles);

Categorical variables representing the subgroups and appropriate interaction terms between the subgroup variables and study treatments will be added to the models evaluating treatment effects on the primary and secondary endpoints (models 1 – 5); a separate set of models will be run for each subgroup analysis. The final models that were developed for each endpoint during the primary analyses will be used as initial models for the subgroup analysis, i.e. if the primary analysis for the endpoint detected no interactions between the study treatments the initial model used for the subgroup analyses for this endpoint will not include interaction terms for the treatments. For simplicity, examples of the subgroup analysis models formulated below assume two sub-groups and no interaction between the study treatments. If the interactions will be detected in primary analyses, appropriate interaction terms will be added to the subgroup analysis

models.Reference coding will be used for categorical variables and a variable with K categories will be represented in the models by K – 1 indicator variables each taking on a value of 1 if the participant belongs to this sub-group and 0 otherwise.

For the subgroup analyses the primary analysis models (1 – 5) will be modified as described below:

Poisson model:

$$Log(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Fall + \beta_5 S + \gamma_1 (S \times VD) + \gamma_2 (S \times PE) + \gamma_3 (S \times \Omega 3) + log(Time) + \varepsilon, \quad (6)$$

where S – a binary indicator variable describing two sub-categories being compared and all other parameters are defined the same way as for model 1. Different effects of study treatments in subgroups will be evaluated by testing hypotheses of $\gamma_1 = \gamma_2 = \gamma_3 = 0$. If this hypothesis is rejected, then each of the interaction terms will be tested separately.

Linear mixed effects model:

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Time_{ij} + \beta_5 Fall_i + \beta_6 Y\_BL_i + \beta_7 S_i + \gamma_1 (S_i \times VD) + \gamma_2 (S_i \times PE) + \gamma_3 (S_i \times \Omega 3) + \gamma_4 (Time_{ij} \times S_i \times VD_i) + \gamma_5 (Time_{ij} \times S_i \times \Omega 3_i) + \gamma_6 (Time_{ij} \times S_i \times PE_i) + b_{1i} + b_{2i} Time_{ij} + \varepsilon_{ij}, \quad (7)$$

where S_i – a binary indicator variable identifying a subcategory to which belongs the i-th participant being compared and all other parameters are defined the same way as for model 2.

Logistic model:

$$Logit(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Fall + \beta_5 S + \gamma_1 (S \times VD) + \gamma_2 (S \times PE) + \gamma_3 (S \times \Omega 3) + \varepsilon \quad (8)$$

Where all the variables are defined the same way as for the model (6) above.

Cox proportional hazards model:

$$h(t, \mathbf{X}) = h_0 t \times exp[\beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Fall + \beta_5 S + \gamma_1 (S \times VD) + \gamma_2 (S \times PE) + \gamma_3 (S \times \Omega 3)] \quad (9)$$

Where all the variables are defined the same way as for the model (6) above.

8.3. Exploratory Efficacy Analyses

8.3.1. Exploratory Endpoints

Exploratory endpoint will be analyzed using the models formulated for the primary and secondary endpoints (models 1 – 5 above) and using the same approach to modeling strategy (see Hypotheses testing strategy for all models on page 38).

Poisson models will be used for the following exploratory endpoints (model 1):

- Incidence rate of any cardiovascular events
- Incidence rate of implant infections after total hip or knee replacement due to fracture or osteoarthritis
- Incidence rate of gastro-intestinal infections

Risk of binary exploratory endpoints will be analyzed using logistic models (model 3) and time to event will be evaluated with Cox proportional hazards model (model 4). The following endpoints are classified as binary:

- Repeated fracture
- Sarcopenia
- Myocardial infarction
- Stroke
- Congestive heart disease
- Cardiovascular mortality
- Dimentia
- Any cancer
- Gastro-intestinal cancer
- Breast cancer (in women)
- Prostate cancer (in men)
- Incident symptomatic knee osteoarthritis
- Incident symptomatic hip osteoarthritis
- Incident symptomatic hand osteoarthritis
- Composite endpoint: incident symptomatic knee, hip, or hand osteoarthritis

Changes in continuous or interval exploratory endpoints will be compared using linear mixed effects models (model 2). Continuous endpoints are listed below:

- Decline in physical activity
- Severity of hip pain in participants with prevalent symptomatic hip osteoarthritis
- Severity of hand pain in participants with prevalent symptomatic hand osteoarthritis

Categorical secondary endpoints will be analyzed using an ordinal logistic regression will be built for 12-, 24-, and 36-month time point. The proportional hazards assumption will be checked for each model, if the assumption does not hold, the model will be re-formulated as a multinomial logistic regression models.

8.3.2. Ancillary Fracture Healing Study

Primary endpoint: clinical fracture healing as evaluated by PROMIS-HAQ score at 6, 12, and 18 weeks after the fracture. The endpoint will be defined as two variables: (1) absolute change in PROMIS-HAQ score between the last known pre-fracture score and the score at 6, 12, and 18 weeks after the fracture and (2) a binary variable which takes on a value of 1 if the PROMIS-HAQ score at 6, 12, and 18 weeks after the fracture is no more than 10% lower than the last know pre-fracture score and 0 otherwise. The continuous endpoint will be analyzed with a linear mixed effects model:

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$$PHS_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Time_{ij} + \beta_5 Fall_i + \beta_6 PHS\_Prior_i + \beta_7 Region_i + \gamma_1(VD_i \times \Omega 3_i) + \gamma_2(VD_i \times PE_i) + \gamma_3(\Omega 3_i \times PE_i) + \gamma_4(VD_i \times \Omega 3_i \times PE_i) + \gamma_5(Time_{ij} \times VD_i \times \Omega 3_i) + \gamma_6(Time_{ij} \times VD_i \times PE_i) + \gamma_7(Time_{ij} \times \Omega 3_i \times PE_i) + \gamma_8(Time_{ij} \times VD_i \times \Omega 3_i \times PE_i) + b_{1i} + b_{2i} Time_{ij} + \varepsilon_{ij} \quad (10)$$

Where:

PHS_{ij} – post-fracture PROMIS-HAQ score for the i-th participant and time j;
$Time_{ij}$ – time since the fracture;
PHS_Prior_i – last known pre-fracture PROMIS-HAQ score;
$Region_i$ – fracture region (arm vs. leg);
All other variables are defined the same way as for model 2.

The binary endpoint will be evaluated witha marginal logistic GEE model formulated identically to model (5). Model fit diagnostic procedures will be carried out the way is is described for model (5).

8.4. Per Protocol Analyses

Per protocol analyzes will be performed using the same methods and models as for corresponding intention-to-treat analyses, but in the study population that complied with the assigned study treatment.

- A participant who complied with the assigned vitamin D / omega-3 supplementation is defined as the participant who consumed at least 80% of the study medication capsules.
- A participant who complied with the assigned physical exercise program is defined as the participant who reported performing assigned physical exercisesregimenat least once per week in 80% of the time.

9. Safety Analyses

Incidence of adverse events within treatment groups will be analyzed both overall and stratified by severity (serious vs. not serious) and relationship to the study treatments (related vs. not related). When calculating the incidence of adverse events each participant will only be counted once and any repetition of the same adverse event within the participant will be ignored; the denominator will be the total population size.

In addition incidence of hypercalcemia and kidney stones in the participants supplemented with vitamin D will be compared against that in the participants that received placebo. Omega-3 fatty acids supplementation strategies (active supplementation vs. placebo) will be compared with respect toincidence of GI symptoms, skin abnormalities, and bleeding.

10.Reporting Conventions

P-values ≥ 0.001 will be reported to 3 decimal places, p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, quantiles, and any other statistics will be reported to 2 decimal places. Estimated regression coefficients will be reported to 3 significant figures.

11.Technical Details

All analyses will be performed centrally by the data management team at the DO-HEALTH coordinating center using SAS statistical software v9.2 (Copyright© 2002-2003 by SAS Institute Inc., Cary, NC, USA) or a later version if it is available at the time of analysis. All SAS codes needed to perform the analyses will be developed beforehand and stored both as electronic files and as printouts attached to this Statistical Analysis Plan. To ensure quality, all SAS codes will be run on a simulated data set and reviewed by DO-HEALTH Head Biostatistician.

12.Listing of Tables, Listings and Figures

I will come up with these once I have run the analysis on a simulated data set.

13. Endpoint variables data locations in DO-HEALTH CRFs.

Endpoint	Time point	CRF	CRF items
Primary endpoints			
Incidence of non-vertebral fractures	Baseline Phone calls Clinical visits		

14.References

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Appendix. Endpoint Descriptions.

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
Primary endpoints				
Incidence of non-vertebral fractures (only 1 fracture per person)	3-monthly phone calls	F201, Q.3 and F913, Q.2		Only the first non-vertebral fracture per participant is counted for this endpoint. The endpoint variable takes on two values: 1 – "had at least 1 non-vertebral fracture over the period of follow up" or 0 – "had no non-vertebral fractures over the period of follow up"
	Yearly follow up clinical visits	F304, Q.2 and F913, Q.2		
Functional decline	Baseline	F108, SPPB section (QQ. 1 – 5)		SPPB scoring algorithm is available at ../Dropbox (ZAuM)/DO-HEALTH CT/Statistical Analyses/Scoring Algorithms or at http://geriatrictoolkit.missouri.edu/ShortPhysicalPerformanceBattery.pdf The endpoint variable is defined as total SPPB score measured at 1, 2, and 3 years of follow up.
	Yearly follow up clinical visits	F308, SPPB section (QQ. 1 – 5)		
Change in systolic and diastolic blood pressure	Baseline	F105, Q.7		The endpoint variable is calculated at 1-, 2-, and 3-year time points and is defined as an average of the 2nd and the 3rd measurements of systolic (diastolic) blood pressure at each of the time points.
	Yearly follow up clinical visits	F305, Q.7		
Cognitive decline	Baseline	MoCA Questionnaire form Total Score		The standard MoCA questionnaire form includes a scoring algorithm and provides a total MoCA score. The endpoint is defined as total MoCA score measured at 1, 2, and 3 years of follow up.
	Yearly follow up clinical visits			
Incidence of infections	3-monthly phone calls	F201, Q.1		The endpoint variable is defined as a total number of infections that a participant had during the entire 3-year follow up period (or the entire period that the person participated in the study).
	Yearly follow up clinical visits	F304, Q.7		
Secondary endpoints				
Incidence of hip fractures	3-monthly phone calls	F201, Q.3 and F913, QQ.2, 3		For this endpoint only the fractures that have "Hip" indicated as a fractured bone in F913 Q.2 are selected. Pathologic hip fractures (the

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
	Yearly follow up clinical visits	F304, Q.2 and F913, QQ.2, 3		fractures that have "Spontaneous / pathologic fracture" selected in F913 Q.3 are excluded from this endpoint. The endpoint variable is defined as a total number of non-pathologic hip fractures that a participant had during the entire 3-year follow up period (or the entire period that the person participated in the study).
Incidence of vertebral fractures	Yearly follow up clinical visits	F913, Q.2 and DXA report		For this endpoint only the fractures that have "Vertebra(e)" indicated as a fractured bone in F913 Q.2 are selected. Vertebral fractures have to be confirmed by a DXA report at one of the clinical visits. The endpoint variable is defined as a total number of vertebral fractures that a participant had during the entire 3-year follow up period (or the entire period that the person participated in the study).*This endpoint is assessed only in participants evaluated by DXA equipped centers: Zurich, Berlin, Toulouse, and Coimbra.*
Incidence of any fractures	3-monthly phone calls	F201, Q.3 and F913, Q.3		For this all non-pathologic fractures are selected. Pathologic hip fractures (the fractures that have "Spontaneous / pathologic fracture" selected in F913 Q.3 are excluded from this endpoint. Vertebral fractures need to be confirmed by a DXA report as described above. The endpoint variable is defined as a total number of non-pathologic fractures that a participant had during the entire 3-year follow up period (or the entire period that the person participated in the study). *This endpoint is assessed only in participants evaluated by DXA equipped centers: Zurich, Berlin, Toulouse, and Coimbra.*
	Yearly follow up clinical visits	F304, Q.2 and F913, Q.3		
Bone mineral density (BMD) at the spine and hip	Yearly follow up clinical visits	None		Spine BMD is defined as BMD of L1 – L4 vertebral bodies; hip BMD is defined as femoral neck BMD (average between the left and right leg measurements). The endpoint variables are defined as spine or h
				at 1, 2, and 3 years of follow up.
Number of any low trauma falls	3-monthly phone calls	F201, Q.2 and F912 QQ.2 – 7		

Commented [ES26]: The data come directly from DXA in electronic form. Once the database is formed, the appropriate variable names should be plugged in here.

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
	Yearly follow up clinical visits	F304, Q.1 and F912 QQ.2 – 7		The endpoint variable is defined as a total number of low trauma falls that a participant had during the entire 3-year follow up period (or the entire period that the person participated in the study).
Number of injurious falls	3-monthly phone calls	F201, Q.2 and F912 QQ.2 – 7		For this endpoint only those low trauma falls that lead to an injury (answer "Yes" to F912, Q.7) are selected. The endpoint variable is defined as a total number of injurious falls that a participant had during the entire 3-year follow up period (or the entire period that the person participated in the study).
	Yearly follow up clinical visits	F304, Q.1 and F912 QQ.2 – 7		
Number of participants who fell	3-monthly phone calls	F201, Q.2		The endpoint variable is defined as a total number of participants who sustained at least 1 low-trauma during each 3-month period between the two subsequent contacts. Participants that sustained more than 1 low-trauma fall during a 3-month period should only be counted once for that period.
	Yearly follow up clinical visits	F304, Q.1		
Reaction time	Baseline	F108, Q.6		This endpoint is recorded directly and defined as the time (in seconds) it took a participant to complete 5 stand-ups from a chair.
	Yearly follow up clinical visits	F308, Q.6		
Grip strength	Baseline	F108, QQ.9, 10		This endpoint is defined an average of grip strength from the three recorded attempts in each hand.
	Yearly follow up clinical visits	F308, QQ.9, 10		
Muscle mass in the upper and lower extremities (appendicular lean muscle mass)	Baseline			This endpoint is measured directly by DXA and is defined as a sum of muscle mass of both arms and legs.
	Yearly follow up clinical visits			
Musculoskeletal pain	Baseline	McGill pain map		This endpoint is defined as a number of painful regions at each time point.
	Yearly follow up clinical visits			
Dual tasking gait speed	Baseline	F108, QQ.11, 12		

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
	Yearly follow up clinical visits	F308, QQ.11, 12		This endpoint is defined as a difference between two gait speeds: single task gait speed V_{ST} = 10 / Time recorded in Q.11 and dual task gait speed V_{DT} = 10 / Time recorded in Q.12. The difference: $\Delta = V_{DT} - V_{ST}$.
Incident hypertension	Yearly follow up clinical visits	F305, Q.7; Diagnoses page (F104, F904C)		For this endpoint only incident cases of hypertension should be counted. Hypertension of this endpoint is defined as: (1) blood pressure (average of the 2nd and 3rd measurements) is at or above 140/90 mmHg AND / OR (2) a diagnosis of hypertension (ICD-10 codes I10 – I15) established by a primary case or other physician and recorded in the Diagnoses table (F104, F904C). All cases of incident hypertension have to be confirmed by the Independent Physician Endpoint Committee. This endpoint is defined as a total number of participants who have been diagnosed with hypertension during the entire 3-year follow up period. A participant who had multiple cases of hypertension should be counted once for this endpoint.
Mental health decline	Baseline	MoCA Questionnaire		For this endpoint only the total MoCA score located at the bottom of the form will be used. A score measured at each follow up clinical visit (at 1, 2, and 3 years) is compared to the baseline score. The endpoint is defined as: (1) a binary variable where mental health decline is considered to have occurred if a MoCA score at a follow up visit is lower than the baseline MoCA score; (2) a difference between each follow-up score and the baseline score, i.e.: for visit 1: $MoCA_{DIFF} = MoCA_1 - MoCA_{BL}$.
	Yearly follow up clinical visits			
Incidence of depression	Yearly follow up clinical visits	Geriatric depression scale (GDS) short form		Scoring algorithm for GDS short form is located at ../Dropbox (ZAuM)/DO-HEALTH CT/Statistical Analyses/Scoring Algorithms. For this endpoint depression is defined as GDS score of 10 or more. The endpoint is defined as total number of participants who scored 10 or more on GDS at least once during the 3-year follow up period (or the entire period that the person participated in the study). Participants who had depression more than once should be counted once for this endpoint.
	Baseline			

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
Dual tasking gait variability	Yearly follow up clinical visits			This endpoint (coefficient of variability) is calculated from the step time mean and standard deviation parametersthat aremeasured directly by the GAITRite® system. The coefficient of variability is calculated as CoV = [SD/mean] × 100.
Number of any upper respiratory infections, flu-like illnesses, and severe infections that lead to hospital admission	3-monthly phone calls Yearly follow up clinical visits	F914, QQ. 3 and 6. F910, Q. 8.		All incident infection are recorded in F914 (one form per infection). The form provides information about infection type (upper respiratory, sinusitis, etc.) and dates when the infections occurred. Further information including the exact diagnosis and ICD-10 code can be found in F910 with matching date. For this endpoint all number of any upper respiratory infections, flu-like illnesses, and severe infections that lead to hospital admission that occurred in a participant during follow up and are confirmed by the Independent Physician Endpoint Committee are counted.
Severity of knee pain in patients with symptomatic knee OA at baseline	Baseline Yearly follow up clinical visits	F106 Knee OA checklist, KOOS Pain subscale KOOS Pain subscale		This endpoint is evaluated only in participant who were diagnosed with symptomatic knee osteoarthritis at (or before) baseline. Diagnosis of knee OA at baseline is confirmed by and affirmative answer to the knee OA checklist in F106. Pain intensity is quantified by KOOS pain subscale (P1 – P9) as using the following formula: $PAIN = 100 - \frac{Mean\ Score\ (P1-P9)\times 100}{4}$. Individual scale items are assigned consecutive scores from 0 = None to 4 = Extreme (see official KOOS scoring manual).
Rate of knee buckling in patients with symptomatic knee OA at baseline	Baseline Yearly follow up clinical visits	F106 Knee OA checklist, Knee Buckling Questionnaire (Q.3) Knee Buckling Questionnaire (Q.3)		This endpoint is evaluated only in participant who were diagnosed with symptomatic knee osteoarthritis at (or before) baseline. Diagnosis of knee OA at baseline is confirmed by and affirmative answer to the knee OA checklist in F106. The endpoint is assessed by question 3 of the Knee Buckling Questionnaire. The question has 5 categories of answers evaluating the number of time participant's knees buckled during the past 3 months: once, 2 – 5, 6 – 10, 11 – 24, and 24+ times. This endpoint is analyzed as a categorical variable at 12, 24, and 36 months.

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
Total number of painful joints	Baseline	Joint Map (homunculus)		Total number of painful joints indicated by a participant on the map at each time point is recorded for this endpoint.
	Yearly follow up clinical visits	Joint Map (homunculus)		
NSAID use due to knee pain	Baseline	F106, Q.3		This endpoint is defined as a binary (yes/no) variable at each time point.
	Yearly follow up clinical visits	F306, Q.3		
Oral health decline	Baseline	GOHAI Questionnaire Score		The final GOHAI score ranges from 0 (worst oral health) to 60 (best oral health).The score is calculated for each time point: baseline, 12, 24, and 36 months of follow up.
	Yearly follow up clinical visits			
Tooth loss	Baseline	F105 or F105ZH, Q.9 - 12.		Total number of teeth (own and prosthetic) present and missing is recorded. In addition the number of fixed or removable prostheses is recorded.
	Yearly follow up clinical visits	F305 or F305ZH, Q.8 - 11.		
Number of any new gastro-intestinal symptoms	Baseline	ROME III questionnaire (symptom-specific questions)		The endpoint is evaluated as a total number of **NEW** gastrointestinal symptoms reported by the participant since the previous clinical visit.
	Yearly follow up clinical visits			
	Baseline			Insulin sensitivity is calculated using QUICKI

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
Insulin sensitivity and beta cell function	Yearly follow up clinical visits	Lab measurement sheets: fasting glucose and fasting insulin		$OUICKI = \frac{1}{log(fasting\ insulin, \mu U/mL) + log(fasting\ glucose, mg/dL)}$ Insulin resistance and β-cells function is calculated using HOMA index: $HOMA_{IR} = \frac{Glucose, \frac{mmol}{L} \times Insulin}{22.5}$ $HOMA_{\beta} = \frac{20 \times Insulin}{Glucose, \frac{mmol}{L} - 3.5}$
Body composition and fat mass	Baseline Yearly follow up clinical visits	DXA measurements (electronic database)		Fat mass is estimated directly by the DXA machine. Body composition measurements (% bone, % fat, and % soft tissue) are measured directly by DXA machine and stored in an electronic database
Kidney function	Baseline Yearly follow up clinical visits	F103, Q.3. F303, Q.4.		
Quality of life	Baseline 6-, 18-, and 36-month phone calls Yearly follow up clinical visits	EQ5D-3L questionnaires;		TTO index SAS algorithms for Germany, France, and Spain available from are available upon request from EuroQol Executive Office (userinformationservice@euroqol.org)

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
Frailty level	Baseline	Weight: F102, Q.5. Exhaustion: SHARE-FI, Q.1. Physical activity: NHS questionnaire Walk time: F108, Q.4. Grip strength: F108, Q.10.		Frailty will be defined according to Linda Fried criteria.
	Yearly follow up clinical visits	Weight: F304 Exhaustion: SHARE-FI, Q.1. Physical activity: NHS questionnaire Walk time: F308, Q.4. Grip strength: F308, Q.10.		
Level of activities of daily living	Baseline Yearly follow up clinical visits	PROMIS-HAQ		Participants' responses are to be converted into HAQ score as per scoring instruction published by Stanford University School of Medicine (19)
Incident mortality, incident permanent nursing home admissions, and the number of acute hospital admissions		F910, Q.10.		
Exploratory endpoints				

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Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
Incidence of repeat fractures	3-monthly follow-up phone calls	F201, Q.3.		This endpoint is evaluated only in participants who already had one fracture during the follow up period. The endpoint is defined as a second fracture that occurred in a participant at an endpoint different form the first fracture. The two fractures can occur during the same 3-month period just not at the same day.
	Yearly follow up clinical visits	F304, Q.2.		
Incident disability regarding activities of daily living	Baseline	PROMIS-HAQ		Calculated HAQ score will be converted into a binary variable: 0 – maintained or improved the score since the previous time point; 1 – the score worsened since the previous time point.
	Yearly follow up clinical visits			
Incidence of sarcopeina	Baseline	Height: F102, Q.4. Appendicular muscle mass: DXA electronic database SPPB, hand grip strength: F108, F108B		At the time of writing there is no consensus as to the definition of sarcopenia. The data suggested here are the most likely ones to be used to define sarcopenia.
	Yearly follow up clinical visits	Height: F302 Appendicular muscle mass: DXA electronic database SPPB, hand grip strength: F308, F308B		

Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
Incident frailty	Baseline	Weight: F102, Q.5. Exhaustion: SHARE-FI, Q.1. Physical activity: NHS questionnaire Walk time: F108, Q.4. Grip strength: F108, Q.10.		Frailty will be defined according to Linda Fried criteria.
	Yearly follow up clinical visits	Weight: F304 Exhaustion: SHARE-FI, Q.1. Physical activity: NHS questionnaire Walk time: F308, Q.4. Grip strength: F308, Q.10.		
Physical activity level	Baseline Yearly follow up clinical visits	Nurses' Health Study Questionnaire (excerpt)		Energy expenditure can be estimated from physical activity reported by a participant using Compendium of Physical Activities (https://sites.google.com/site/compendiumofphysicalactivities/)
Incidence of major cardiovascular events		F910, Q.8.		
Incidence of dementia	Yearly follow up clinical visits	MoCA questionnaire		MoCA score
Incidence of cancer	3-monthly phone calls	F201, Q.4. and F910 Q.8.		

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Endpoint	Time point	CRF location	DO-HEALTH database variable(s)	How the endpoint is assessed
	Yearly follow up clinical visits	F304, Q.3. and F910 Q.8.		F201, Q.4 and F304, Q.3 can be used for estimating cancer incidence. F910 Q.8 contains and ICD-10 code which can be used for establishing the type of cancer.
Number of implant infections after total hip or knee replacement due to fracture or osteoarthritis	3-monthly phone calls Yearly follow up clinical visits	F914, Q.7		
Number of gastro-intestinal infections	3-monthly phone calls Yearly follow up clinical visits	F914, Q.7; F910 Q.8.		F910 Q.8.contains and ICD-10 code which can help in establishing that the infection was a gastrointestinal one.
Incidence of symptomatic knee osteoarthritis	Yearly follow up clinical visits	F306 (knee OA checklist), KOOS		Only the first diagnosis of symptomatic knee OA is counted for this endpoint
Incidence of symptomatic hip osteoarthritis	Yearly follow up clinical visits	F306 (hip OA checklist), HOOS		Only the first diagnosis of symptomatic hip OA is counted for this endpoint
Incidence of symptomatic hand osteoarthritis	Yearly follow up clinical visits	F306 (hand OA checklist), QuickDASH		Only the first diagnosis of symptomatic hand OA is counted for this endpoint
Severity of hip pain in those with prevalent symptomatic hip OA	Yearly follow up clinical visits	HOOS Pain Section (P1 – P9)		
Severity of hand pain in those with prevalent symptomatic hand OA	Yearly follow up clinical visits	QuickDASH Q.9.		

DO-HEALTH statistical analysis plan and definitions of the variables

Version 1.5 dated 03.01.2018

This document "statistical analysis plan Do-health" was developed by Dr. Patricia Chocano-Bedoya (Epidemiologist, Centre on Aging and Mobility, University Hospital Zurich) and Prof. Heike A. Bischoff-Ferrari (PI DO-HEALTH), under supervision and with final edits by Prof. E. John Orav (Prof. of Biostatistics, Harvard Medical School, and Head biostatistician of DO-HEALTH). The statistical methods final revision was on 11.12.2017 and final editorial changes were incorporated by 03.01.2018.

The version 1.5 is the final version implemented in DO-HEALTH data analyses of all protocol defined endpoints.

No analyses on the DO-HEALTH trial data set and with regards to the interventions were performed prior to the finalization of the DO-HEALTH analysis plan as outlined in this document.

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Abbreviations and definitions

ACR	American College of Rheumatology
ALT	Alanine Aminotransferase
AST	Asparagine Aminotransferase
BMD	Bone Mineral Density
CoV	Coefficient of Variability
CRF	Case Report Form
CRP	C-reactive Protein
DHA	Docosahexaenoic Acid
DSMB	Data Safety and Monitoring Board
DXA or iDXA	Dual-energy X-ray Absorptiometry
EPA	Eicosapentaenoic Acid
FRAX®	WHO Fracture Risk Assessment Tool
GEE	Generalized Estimating Equation
GI	Gastro-intestinal
HDL	High Density Lipoprotein
HOOS	**Hip** injury and Osteoarthritis Outcome Score
Incidence	means that an individual has the event or not
ITT	Intention to Treat
KOOS	Knee injury and Osteoarthritis Outcome Score
LDH	Lactate Dehydrogenase
MAR	Missing at Random
MMSE	Mini Mental State Examination
OA	Osteoarthritis
PI	Principal Investigator
PUFA	Polyunsaturated Fatty Acid
SD	Standard Deviation
SPPB	Short Physical Performance Battery
TSH	Thyroid Stimulating Hormone

1. Study design and objectives

1.1. Study design

DO-HEALTH is a randomized, double-blind, placebo-controlled, 2×2×2 factorial, multi-center clinical trial. The trial is performed at 7 recruitment centers located in 5 countries: Switzerland (University of Zurich, Basel University Hospital, Geneva University Hospital), France (University of Toulouse Hospital Centre), Germany (Charité Berlin), Portugal (University of Coimbra), and Austria (Innsbruck Medical University).

1.2. Study objectives

Main objectives:

- To improve healthy ageing in European seniors
- To reduce healthcare costs via the implementation of effective and broadly applicable disease prevention interventions

Specific Objectives:

- To establish whether vitamin D, omega-3 fatty acids, and a simple home exercise program will prevent disease at older age
- To assess comparative effectiveness and cost-benefit of the interventions

2. Endpoints

2.1. Primary endpoints

Bone	Incidence of non-vertebral fractures
Muscle	Functional decline
Cardiovascular	Change in systolic and diastolic blood pressure
Brain	Cognitive decline
Immunity	Number of infections of any type

2.2. Secondary endpoints

Bone	Incidence of hip fractures
	Incidence of vertebral fractures
	Incidence of any fractures
	Bone mineral density (BMD)at the spine and hip
Muscle	Number of any low trauma falls
	Number of injurious falls
	Number of participants who fell
	Reaction time
	Grip strength
	Muscle mass in the upper and lower extremities
	Musculoskeletal pain
	Dual tasking gait speed

Cardiovascular	Incidence of hypertension over 36 month
Brain	Mental health decline
	Incidence of depression
	Dual tasking gait variability
Immunity	Number of any upper respiratory infections, flu-like illnesses, and severe infections that lead to hospital admission over the 36-month period
Bone/cartilage	Severity of knee pain in patients with symptomatic knee OA at baseline
	Rate of knee buckling in patients with symptomatic knee OA at baseline
	Total number of joints with pain
	NSAID use due to knee pain
Dental	Decline in oral health
	Tooth loss
Gastro-intestinal	Number of any new gastro-intestinal symptoms
Glucose-metabolic	Fasting blood concentration of glucose and insulin
	Body composition and fat mass
Kidney	Decline in kidney function
Global health	Quality of life
	Frailty level
	Level of activities of daily living
	Incident mortality, incident permanent nursing home admissions, and the number of acute hospital admissions

2.3. Biomarker endpoints

Bone	calcium, phosphate, 25(OH)D, intact PTH, urinary calcium/creatinine ratio (second spot urine), Beta-Crosslaps serum, P1NP
Cardiovascular	Troponin T, NT-proBNP, homocysteine, CK, cholesterol, HDL-cholesterol, triglycerides
Inflammation	CRP, IL6
Gastro-intestinal	AST, ALT, gGT, alkaline phosphatase, bilirubin
Glucose-metabolic	fasting glucose, insulin
Kidney	serum creatinine; calcium/creatinine ratio in second spot urine, serum urea, uric acid
Global Health	Ions: sodium, potassium, chloride, magnesium Proteins: total protein, albumin, ferritin, soluble transferrin receptor Hormones: TSH, fT4, fT3, cortisol Vitamins: folic acid, vitamin B12, 25(OH)D
Adherence to treatment	serum 25(OH)D, plasma PUFA concentrations (EPA, AA, DPA, DHA).
Bone (novel)	sclerostin
Muscle (novel)	myostatin
Inflammation (novel)	TNF-α, IL-10, IL-17, IL-22, IL-8*, IL-6*, IL-1β*
Cellular immunity (novel)	CD3, CD4, CD25, CD127

* not included in the current protocol

2.4. Exploratory endpoints

Bone	Incidence of repeat fractures
Muscle	Incident disability regarding activities of daily living
	Incident sarcopenia
	Incident frailty
	Level of physical activity
Cardiovascular	Incidence of major cardiovascular events
Brain	Incidence of dementia
Immunity	Incidence of cancer
	Number of implant infections after total hip or knee replacement due to fracture or osteoarthritis
	Number of gastro-intestinal infections
Bone/Cartilage-Arthritis	Incidence of symptomatic osteoarthritis
	Incident symptomatic hip osteoarthritis
	Incident symptomatic hand osteoarthritis
	Incident symptomatic knee
	Incident symptomatic knee, hip or hand osteoarthritis
	Severity of hand pain in those with prevalent symptomatic hip osteoarthritis
	Severity of hand pain in those with prevalent symptomatic hand OA

2.5. Ancillary Fracture healing study

Primary	Rate of clinical fracture healing
Secondary	Rate of functional fracture healing
Exploratory	Rate of radiological fracture healing

3. Screening

3.1. Screening Procedures

After a prospective participant had read the DO-HEALTH information and placed a call to the local DO-HEALTH telephone hotline, a trained study nurse conducted a pre-screening interview and scheduled a screening visit at the respective site for those who fulfilled the pre-screening criteria (see 'PreScreen' in 3.2)

At the screening visit, participants signed the informed consent form, completed the MMSE and had a blood test for calcium and creatinine levels. To ensure that also the minimal mobility function was met a screening walk and a chair test were performed with the prospective participant by the study nurse. Final eligibility of the prospective participant was assessed by the study MD and was depended on the answers to the inclusion /exclusion criteria, blood levels for serum calcium and creatinine, cognitive (MMSE ≥ 24) and physical function (screening walk and chair test) and the ability to swallow the study capsules.

3.2. Inclusion/Exclusion criteria

3.2.1. Inclusion criteria

- Age ≥ 70 years (PreScreen)
- Mini Mental State Examination Score of at least 24
- Living in the community (PreScreen)
- Sufficiently mobile to reach the study centre
- Able to walk 10 meters with or without a walking aid and getting in and out of a chair without help (PreScreen)
- Able to swallow study capsules
- Able and willing to participate, sign informed consent (including consent to analyze all samples until drop-out or withdrawal) and cooperate with study procedures

3.2.2. Exclusion criteria

- Consumption of more than 1000 IU vitamin D/day in the 3 months prior to enrollment, or unwillingness to reduce to 800 IU/d of vitamin D (current standard of care) for the duration of the trial (PreScreen)
    i. 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, may be enrolled after a 3-month wash-out period where the maximum daily intake is limited to 800 IU of vitamin D
    ii. Provision 2: an individual who consumed an average vitamin D dose higher than 2000 IU/day in the 3 months prior to enrollment, may be enrolled after a 6-month wash-out period where the maximum daily intake is limited to 800 IU of vitamin D
- Unwilling to limit calcium supplement dose to a maximum of 500 mg per day for the duration of the trial (PreScreen)
- Taking omega-3 fat supplements in the 3 months prior to recruitment and or unwilling to refrain from the use of omega-3 supplements for the duration of the trial (PreScreen)
- 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

- Current or recent (previous 4 months) participation in another clinical trial, or plans of such participation in the next 3 years (PreScreen)
- Presence of the following diagnosed health conditions in the last 5 years:
    i. History of cancer (except non-melanoma skin cancer)
    ii. Myocardial infarction, stroke, transient ischemic attack, angina pectoris, or coronary artery intervention
- Severe renal impairment (creatinine clearance ≤ 15 ml/min) or dialysis
- Hypercalcaemia (> 2.6 mmol/l)
- Hemiplegia or other severe gait impairment
- History of hypo- or primary hyperparathyroidism (PreScreen)
- History of granulomatous diseases (i.e. tubercolosis, sarcoidosis)
- Severe liver disease (PreScreen)
- Major visual or hearing impairment or other serious illness that would preclude participation (PreScreen)
- Living with a partner who is enrolled in DO-HEALTH (i.e. only one person per household can be enrolled) (PreScreen)
- Living in assisted living situation or in nursing home (PreScreen)
- Temporary exclusion: acute fracture in the last 6 weeks
- Epilepsy and/or use of anti-epileptic drugs
- Individuals who fell more than 3 times in the last month
- Osteodystrophia deformans (Paget's disease)
- For study centers in Germany only: persons who are institutionalized / in prison by court order (§40, Abs. 1, Art. 4, "Gesetz über den Verkehr mit Arzneimitteln").

4. Power

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

Power considerations for the primary endpoints.[*]

Endpoint	Minimum detectable difference[†]	Standard Deviation	Alpha	Power with no interact., N = 1806 (903 + 903)	Power with interaction, N = 902 (451 + 451)
Bone:[‡]					
Incidence rate of any non-vertebral fracture including hip fractures (proportion)	0.073[¶]	–	0.01	0.99	0.80
Muscle:[**]					
lower extremity function (mean SPPB score)	0.40	1.41	0.01	0.99	0.97
Cardiovascular:[**]					
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
Brain:[**]					
Cognitive function (mean MMSE score)	0.7	2.3	0.01	0.99	0.90
Immunity:[††]					
Incidence rate of any infection	0.32	$s_1 = 1.46$ $s_2 = 1.35$	0.01	0.99	0.90

[*] All assumptions about means and standard deviations of the parameters are based on the results of pilot trials to DO-HEALTH (9, 25, 26) and research of other authors(27-29).
[†] Minimum detectable difference was assumed as a minimum difference that is clinically significant.
[‡] Power calculations are based on the Z-test for difference between two proportions.
[¶] Estimated baseline prevalence of any non-vertebral fractures incidence rate / IR = 0.14.
[**] Power calculations are based on the two-sample t-test assuming equal variances.
[††] Power calculations are based on Poisson regression assuming non-equal variances.

5. General Principles for Data Analyses (adapted from protocol)

All analyses plans will be pre-defined and reviewed by the PI, the DO-HEALTH epidemiologist, and the head biostatistician (Prof. E. John Orav). All necessary procedures for the analyses plans and preparation of datasets will be coded in SAS statistical language and stored as electronic SAS files in the designated storage folders. An analysis log-book with a list of programs used per paper will be prepared for all analyses to be run for baseline comparisons and when the trial is finished.

The epidemiology team at the DO-HEALTH coordinating centre will conduct all statistical analyses using SAS v9.4 statistical software (Copyright© 2004by SAS Institute Inc., Cary, NC, USA) and supplemented by diagnostic techniques to assess the adequacy of statistical assumptions. Since the study assesses 5 primary endpoints, a Bonferroni adjustment for multiple comparisons will be applied in the statistical analysis so that $p < 0.01$ is required for statistical significance. In analysis for all other endpoints, a p-value of 0.05 (two-sided) will be considered a threshold for statistical significance.

Baseline analysis: The first analysis will compare baseline characteristics by randomized treatment assignment to ensure that balance was achieved by the randomization. Characteristics to be examined include known risk factors for the primary endpoints, including age, gender, BMI, smoking, alcohol use, physical activity, comorbidity, baseline blood pressure, baseline cognitive function (MMSE and MoCA score), baseline lower extremity function (SPPB score), prevalence of prior fall, and baseline 25(OH)D and omega-3 fatty acid blood levels. Baseline characteristics will be summarized by treatment group, where continuous variables will be presented as means and standard deviations and categorical variables will be presented as frequencies.

Analysis of treatment effects: In this 2×2×2 factorial design, the primary aim is to compare the main effects of **intention-to-treat** with vitamin D, omega-3 fats, and the home exercise program on the 5 primary endpoints as detailed in section 6.

5.1.1. Treatment interactions

We will assess the effect of one treatment while adjusting for the other treatments, also testing for a potential interaction effects between treatments in a two-stage process. First, we will compare a main effects model of vitamin D, omega-3 fatty acids and the home exercise program without interaction terms for the treatments. In the next step, we will explore the interaction between the treatment effects, using a statistical significance at $\alpha = 0.01$ to be included in the second model.

5.1.2.Confounding

The stratification variables for randomization included: recruitment center (7 centers), low trauma fall during previous 12 months prior to the randomization day (yes/no), gender, and age (70 – 84 and 85+).We will assess the effect of one treatment while adjusting for the other treatments and for the stratification variables, as well as pre-defined potential confounders (BMI at baseline).

5.1.3. Missing data

- **Missing data on primary endpoints**

Missing data of <1% on any of the 5 primary outcomes will be considered negligible and missing completely at random (MCAR). Under MCAR assumptions, complete-case analysis (listwise deletion) reduces statistical power, but yields unbiased parameter estimates.

Missing data >1% on any of the 5 primary outcomes will be further inspected by a series of Little's MCAR X2 tests selecting on stratified variables. Non-significance suggests that the missing-data mechanism is ignorable and analyses will proceed with complete-case (listwise deletion) and available-case (pairwise deletion) datasets. MCAR significance will be addressed with two approaches to the treatment of missing data, which will enable a missing-model comparison with sensitivity implications on study outcomes. The first treatment of significant-missing data will be the carry-forward method (within-person / cold deck imputation). The second treatment of missing data will be multiple imputation (MI). MI is available in SAS via the 'PROC MI' and 'PROC MIANALYZE'.

This carry-forward and MI approaches will be used for continuous primary (function; blood pressure; and cognition) and secondary outcomes. For binary (non-vertebral fractures) and ordinal (number of infections) outcomes, the models will adjust for time-in-study and no carry-forward or imputation will be used.

6. Primary endpoints

6.1. Incidence of non-vertebral fractures

Background

All fracture events will be confirmed by X-ray reports or by medical records that describe an X-ray report of the fracture or mention the repair of the fracture. The endpoint was assessed every 3 month (4 visits, 9 phone calls) in all participants. Fracture classification cards will be standardized with the US VITAL trial (detailed fracture classification cards will be described in the fracture assessment manual to DO-HEALTH).

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce the incidence of non-vertebral fractures over the 36-month period (i.e., the outcome will be dichotomized so that subject will have either no fracture, or ≥1 fracture.):

Statistical analysis plan

- Main Analysis:

To test the main effects of the study treatments on incidence rate of non-vertebral fractures, we will use **logistic regression** to estimate the odds of having a fracture vs. not having a fracture. We will estimate the odds ratio for each intervention controlling for the other two interventions, age, and gender, faller status and BMI

$$Logit(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + \beta_{14} \log(Time\_in\_Study)$$

Where:

Y – incident non-vertebral fracture (first fracture only)
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age 85

Data dictionary

Variable Name	Definition		Frequencies/Descripts. (baseline)			Dataset/ Path	Questionnaire
		N (missing)	Range (IQR)	N (%)	Mean (SD)		
	MAIN VARIABLES:						
Fx_occurred	Fracture risk (proportion of at-risk population that experiences a fracture over study)	2157 (0)	0-1 (0-0)	254 (11.8)	NA	doh3.fractures_v10	Fracture Form F913
tot_Fx_Cnt	Incident cases (cumulative count of fracture events over study)	2157 (0)	0-4 (0-0)	0: 1903 (88.2) 1: 220 (10.2) 2: 29 (1.3) 3: 4 (0.2) 4: 1 (0.1)	.14 (.40)		Fracture Form F913
Days_To_First_Fx	Onset of first fracture event from study baseline	253 (1902)	13 - 1159 (330-839)	NA	570.19 (309.41)		Fracture Form F913
ftime	Follow-up time: Time to first fracture, until death, drop-out or till end of follow up	TBD					

SAS code for initial analysis / models

```
proc logistic data= doh3.fractures_v10 desc;
   class female (ref='1') faller
         berlin basel coimbra toulouse geneva innsbruck /param=ref  ;
   model Fx_occurred= tx_vitd tx_w3 tx_ex age female faller bmi
         berlin basel coimbra toulouse geneva innsbruck logtime;
run;
```

6.2. Functional decline (Primary endpoint)

Background

Functional decline will be assessed with the Short Physical Performance Battery (SPPB) (252), 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 (252). The SPPB is a brief performance-based test that includes walking speed, repeated chair stands,

and a balance test. Its three components are each scored 0 to 4, with 4 indicating the highest level of performance, and are summed up to yield an overall score. The SPPB was assessed at all clinical visits: at baseline, 12, 24, and 36 months in all participants.

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce functional decline

Statistical analysis plan

- Main analyses:

We will use repeated measurement analyses and will estimate the mean change in SPPB for each intervention using indicators for treatment exposure and time, controlling for the other two interventions, stratification factors (age, and gender, faller status, center) and BMI. We will use the following formula**,** adjusting for unstructured correlation over time using generalized estimating equations:

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Age_i + \beta_5 Gender_i + \beta_6 Fall_i + \beta_7 BMI_i + \beta_{8-13} Centers_i + \gamma_{1-3} Time_{ij} + \gamma_{4-6}(Time_{ij} \times VD_i) + \gamma_{7-9}(Time_{ij} \times VD_i) + \gamma_{10-12}(Time_{ij} \times PE_i) + \varepsilon_{ij}$$

Where:

i – i-th patient (i = 1, 2, …, 2152);
j – j-th visit (j = 0 (baseline), 1 (12 months), 2 (24 months), 3 (36 months));
Y_{ij} – SPPB score for i-th patient at j-th visit;
$Time_{ij}$ – $Follow_1$ up time since baseline for i-th participant at j-th visit
VD_i – vitamin D treatment (2000 IU/day vs. placebo) for the i-th participant;
$\Omega 3_i$ – omega-3 fatty acids treatment (1 g/day vs. placebo) for the i-th participant;
PE_i – physical exercise program (strength vs. mobility) for the i-th participant;
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85

Data dictionary

Variable Name	Definition /Code	Frequencies/Descripts. (baseline)			Dataset/ Path	Questionnaire
		N (missing)	Range (IQR)	Mean (SD)		
MAIN VARIABLES:						
SPPB	Sum of Balance, Gait, Repeatchair scores Code: Sum (of **balance gait repeatchair**)	2150 (7)	3-12 (10-12)	10.87 (1.47)	Doh3.subjectsppb	F105
dSPPB	Change of SPPB over time (T1 to T3 minus baseline)	TBD				
SPPB_bl	Baseline SPPB	2150 (7)	3-12 (10-12)	10.87 (1.47)		
DERIVED FROM:						

balance	Measures ability to so side-by-site stands, semi-tandem stands and tandem stands. If a participant completed the TandemStand, they get 4 points
	
	Code:
	`if tandemStand10sec eq 1 then balance = 4;`
	`else if tandemstandtime ge 3 then balance = 3; *Tandem Stand for 3-9.99 seconds;`
	`else if semitandemStand10sec eq 1 then balance = 2; *Semitandemstand for 10 sec;`
	`else if sideBySideStand10sec eq 1 then balance = 1;`
	`else if tandemStand10sec eq . and tandemstandtime eq . and semitandemstand10sec eq . and sidebysidestand10sec eq . then balance=.;`
	`else balance=0;`
		2150 (7)	0-4 (4-4)	3.80 (0.53)	Doh3	F105

gait	Measures the time required to walk 4 meters at a normal pace (use the best of 2 times)
	
	Code:
	`gait=.;`
	`if mingait=. then gait=0;`
	`else if mingait lt 4.82 then gait=4;`
	`else if mingait lt 6.2 then gait=3;`
	`else if mingait lt 8.7 then gait=2;`
	`else gait=1;`
		2157 (0)	0-4 (4-4)	3.88 (0.43)	Doh3	F105

repeatchair	Measures the time required to perform five rises from a chair to an upright position as fast as possible without the use of arms. Code: `if repeatedchairtesttime =. then repeatchair=0; else if repeatedchairtesttime le 11.19 then repeatchair=4; else if repeatedchairtesttime le 13.69 then repeatchair=3; else if repeatedchairtesttime le 16.69 then repeatchair=2; else repeatchair=1;`	2157 (0)	0-4 (3-4)	3.8 (1.09)	Doh3	F105
ADDITIONAL VARIABLES FROM PHYSICAL TESTS:						
mingait	Best of two attempts Code: `min (of gaitspeedtestone gaitspeedtesttwo)`	2152 (5)	2.05-14.34 (3.03-3.98)	3.62 (.96)	Doh3	F105
meangait	Mean of two attempts Code: `mean (of gaitspeedtestone gaitspeedtesttwo)`	2152 (5)	2.08-17.15 (3.14-4.15)	3.78 (1.03)	Doh3	F105
gaitspeed	Mean time required to walk 1 meter Code: `gaitspeed=4/meangait;`	2152 (5)	.23-1.92 (.97-1.27)	1.12 (.23)	Doh3	F105
maxR	Maximum of grip in right hand Code: max (of gripR1 gripR2 gripR3)	2152 (5)	4-135 (48-70)	59.99 (18.44)	Doh3	F105
maxL	Maximum of grip in left hand Code: max (of gripL1 gripL2 gripL3)	2146 (11)	0-120 (46-70)	57.65 (18.71)	Doh3	F105
grip	Maximum dominant hand (R/L) Code: `if dominanthand='left' then grip=maxL; else grip=maxR;`	2152 (5)	4-135 (48-70)	60.05 (18.50)	Doh3	F105

SAS code for initial analysis / models

```
proc genmod data=data;
   class subjectid tx_vitD tx_w3 tx_ex numvisit female faller
         berlin basel coimbra toulouse geneva innsbruck;
   model SPPB = tx_vitD|numvisit tx_w3|numvisit  tx_ex|numvisit
                age female faller bmi
                berlin basel coimbra toulouse geneva innsbruck
                 /type3 residual;
   repeated subject = subjectid / type=un;
lsmeans tx_vitD *numvisit/cl pdiff;
lsmeans tx_w3 *numvisit/cl pdiff;
lsmeans tx_ex *numvisit/cl pdiff;
run;
```

6.3. Systolic and diastolic blood pressure changes (primary endpoint)

Background

Systolic and diastolic blood pressure changes will be assessed at baseline, 12, 24, and 36 months. Blood pressure will be measured after 5-minute rest in a seated position following a standardized protocol validated in a DO-HEALTH pilot trials.

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce systolic and diastolic blood pressure over 36 months.

Statistical analysis plan

- Main analyses:

We will study the effects of the study treatments on systolic/diastolic blood pressure by **linear mixed models**, as in 5.3

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Age_i + \beta_5 Gender_i + \beta_6 Fall_i + \beta_7 BMI_i + \beta_{8-13} Centers_i + \gamma_1 (Time_{ij} \times VD_i) + \gamma_2 (Time_{ij} \times PE_i) + \gamma_3 (Time_{ij} \times \Omega 3_i) + \varepsilon_{ij}$$

Where:

i – i-th patient (i = 1, 2, …, 2152);
j – j-th visit (j = 1, 2, 3);
Y_{ij} – Change in systolic/diastolic blood pressure for i-th patient at j-th visit;
$Time_{ij}$ – Follow$_1$ up time since baseline for i-th participant at j-th visit;
VD_i – vitamin D treatment (2000 IU/day vs. placebo) for the i-th participant;
$\Omega 3_i$ – omega-3 fatty acids treatment (1 g/day vs. placebo) for the i-th participant;
PE_i – physical exercise program (strength vs. mobility) for the i-th participant;
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);

Data dictionary (Alex, Matt/ R: Patricia)

Variable Name	Definition /Code	Frequencies/Descripts. (baseline)			Dataset/ Path	Questionnaire
		N (missing)	Range (IQR)	Mean (SD)		
MAIN VARIABLES:						
Syst_bl	Average of second and third measurements from predominant arm.	2145 (12)	94-217 (130-155)	143.46 (18.37)	dd.hypertension	F108
Diast_bl	Average of second and third measurements from predominant arm.	2145 (12)	47-113 (69-82)	75.88 (10.96)	dd.hypertension	F108
dSyst	Change in systolic blood pressure over time	TBD				
dDiast	Change in diastolic blood pressure over time	TBD				
ADDITIONAL VARIABLES:						
baselineBp_Arm_left	Predominant arm retained for averaging (measurement #s 2 & 3) BP values.	1041 (0)	0-1	48.2%	dd.hypertension	F108
baselineBp_Arm_right	Predominant arm retained for averaging (measurement #s 5 & 6) BP values.	1120 (0)	0-1	51.9%	dd.hypertension	F108
bp	Recoded into ordinal categories, from 0-5 0=optimal (120/80), 1=normal (130/85), 2=hi-normal (140/90), 3=stage1 (160/100), 4=stage2 (180/110), 5=stage3 (>180/>110)	2145 (12) 173 311 433 839 306 83	0-5 (2-3)	2.49 (1.24) 9.1% 14.4% 20.1% 38.9% 14.2% 3.8%	dd.hypertension	F108

SAS code for initial analysis / models

Similar code as for changes in SPPB:

```
proc mixed data=data;
   class subjectid tx_vitD tx_w3 tx_ex numvisit female faller
         berlin basel coimbra toulouse geneva innsbruck;
   model dSyst = tx_vitD|numvisit tx_w3|numvisit  tx_ex|numvisit
                 age female faller bmi
                 berlin basel coimbra toulouse geneva innsbruck /*oth cov*/
                 /solution residual;
   repeated /subject = subjectid type=un;
lsmeans tx_vitD *numvisit/cl pdiff;
lsmeans tx_w3 *numvisit/cl pdiff;
lsmeans tx_ex *numvisit/cl pdiff;
run;
```

6.4.Cognitive decline

Background

Cognitive decline will be assessed using the Montreal Cognitive Assessment (MoCA) at each of the clinical visits: at baseline, 12, 24, and 36 months. The structure of MoCA is similar to that of the Mini Mental State Examination (MMSE) and was found to be more sensitive than MMSE with respect to mild cognitive impairment. In addition subjective cognitive decline will be assed using the subjective memory complains questionnaire at the 36 months visits in a subset of 802 participants at two recruitment centers (Zurich and Basel).

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will improve cognitive function over 36 months.

Statistical analysis plan

We will study the effects of the study treatments on systolic/diastolic blood pressure by **linear mixed models**, as in 5.3

Data dictionary

Variable Name	Definition /Code	Frequencies/Descripts. (baseline)			Datase t/Path	Question naire
		N (missing)	Range (IQR)	Mean (SD)		
MAIN VARIABLES:						
Moca_bl	Total sum of moca scores corrected by education	2152 (5)	10-30 (24-28)	25.7 (3.4)	Doh3. moca	Moca
dMoCA	Change in moca scores over time	TBD				
ADDITIONAL VARIABLES:						
mocasumscore	Total sum of midscores 1-10 without correction by education	2152 (5)	9-30 (23-28)	25.2 (3.5)	Doh3.moca	Moca
eduyears	Total education years				dodata. f102dem ographi cs	F102
newedupoints	*add one point for an individuals with 12 or less years of education ; if eduyears le 12 then newedupoint=1;				Doh3.moca	Moca
VARIABLES USED FOR SUM OF MOCA SCORES:						
		N (missing)	Range	Freq		
Mocamidscore1	if mocamidscore1 eq . then do; mocamidscore1=sum (of trailMaking copyshape clockcontour clocknumbers clockhands);	2151 (6)	0-5	0:26 1:76 2:150 3:337 4:562 5:1001	Doh3.moca	Moca

Mocamidscore2		2151 (6)	0-3	0:6 1:22 2:161 3:1962		
Mocamidscore3		2152 (5)	0-2	0:119 1:509 2:1524		
Mocamidscore4	`if mocamidscore4 eq . then do;` `mocamidscore4=tapas;`	2152 (5)	0-1	0:260 1:1892		
Substract7		2153 (4)	0-3	0:35 1:72 2:281 3:1765		
Mocamidscore6	`if mocamidscore6 eq . then do;` `mocamidscore6=sum (of repeatsentence1 repeatsentence2 );`	2153 (4)	0-2	0:143 1:440 2:1570		
Mocamidscore7	`if mocamidscore7 eq . then do;` `mocamidscore7=words1minute;`	2152 (5)	0-1	0:857 1:1295		
Mocamidscore8	`if mocamidscore8 eq . then do;` `mocamidscore8=sum (of comparison1 comparison2);`	2153 (4)	0-2	0:100 1:531 2:1522		
Mocamidscore9	`if mocamidscore9 eq . then do;` `mocamidscore9=sum (of word1_3 word2_3 word3_3 word4_3 word5_3);`	2152 (5)	0-5	0:192 1:167 2:356 3:467 4:465 5:505		
mocamidscore10	`if mocamidscore10 eq . then do;` `mocamidscore10=sum (of date month year day place city);`	2153 (4)	0-6	2:2 4:13 5:100 6:2038		

SAS code for initial analysis / models

Same code as in 5.3

```
proc mixed data=data;
   class subjectid tx_vitD tx_w3 tx_ex numvisit female faller
         berlin basel coimbra toulouse geneva innsbruck;
   model dMoCA = tx_vitD|numvisit tx_w3|numvisit  tx_ex|numvisit
                 age female faller bmi
                 berlin basel coimbra toulouse geneva innsbruck
                 /solution residual;
   repeated /subject = subjectid type=un;
lsmeans tx_vitD *numvisit/cl pdiff;
lsmeans tx_w3 *numvisit/cl pdiff;
lsmeans tx_ex *numvisit/cl pdiff;
run;
```

6.5. Rate of infections

Background

Occurrence of infections will be assessed every 3 months (4 clinical visits and 9 phone follow-ups). Upon each contact, the participants will be asked whether any infections with or without fever have occurred and whether and when a vaccination was performed (i.e. flu vaccination). In case a participant has experienced infections, a detailed infection questionnaire developed in two pilot trials to DO-HEALTH will be applied. The questionnaire collects information about symptoms, treatment, MD contact and medical assessments. Every case of infection classified as a serious adverse event and any case of infection in the last three months of a participant's follow-up time will be confirmed by medical records. However, all reported infections, confirmed or not, will be used for the primary outcome.

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce the rate of infections over the 36-month follow-up period.

Statistical analysis plan

For the rate of incident infections, we assume a Poisson distribution for the number of infections, and will use a **Poisson regression** analysis to compare the number of infections for each intervention using indicators for treatment exposure, controlling for the stratification factors (age, gender, center, faller status) and BMI.

$$Log(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + log(Time) + \varepsilon$$

Where:

Y – Rate of incident infections
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
Age – Linear spline for age with a knot at age 85
ε – error

SAS code for initial analysis / models

```
proc genmod data=data;
    class subjectid tx_vitD tx_w3 tx_ex female faller
          berlin basel coimbra toulouse geneva innsbruck /param=ref;
    model infection_inc =  tx_vitD tx_w3 tx_ex age female faller bmi
     berlin basel coimbra toulouse geneva innsbruck
     /dist=poisson link=log offset=exp_time;
estimate "Treatment (Group 1 vs. Control)" Treatment 1 0/exp;
estimate "Treatment (Group 1 vs. Control)" Treatment 0 1/exp;
run;
```

7. Secondary endpoints

7.1. Table of Secondary Endpoints and Suggested Method

Bone	Form	Method	Formula
Time to first fracture	F913	Cox Proportional Hazards	1
Total number of fractures (Incidence Rate)	F913	Poisson regression	2
Incidence of hip fractures	F913		
Cumulative risk of hip fracture	F913	Logistic regression	3
Time to first hip fracture	F913	Cox Proportional Hazards	1
Incidence of vertebral fractures	F913		
Cumulative risk vertebral fracture	F913	Logistic regression	3
Time to first vertebral fracture	F913	Cox Proportional Hazards	1
Bone mineral density (BMD)at the spine and hip	DEXA	Linear mixed models	4
Muscle			
Falls	F912		
Number of any low trauma falls (Incidence Rate)		Poisson regression	2
Number of injurious falls (Incidence Rate)		Poisson regression	2
Number of participants who fell		Logistic regression	3
Reaction time	F108/308	Linear mixed models	4
Grip strength	F108/308	Linear mixed models	4
Muscle mass in the upper and lower extremities	DEXA	Linear mixed models	4
Pain	McGill		
Musculoskeletal pain (total score)		Linear mixed models	4
Presence of pain lower/upper extremities		Logistic regression	3
Dual tasking gait speed	F108/308	Linear mixed models	4
Cardiovascular			
Diastolic Blood Pressure	F105/305	Linear mixed models	4
Incidence of hypertension over 36 month	F105/305	Logistic regression	3
Brain			
Mental health	GDS		
Mental health decline (continuous GDS scores?)		Linear mixed models	4
Incidence of depression		Logistic regression	3
Dual tasking gait variability	F108/308	Linear mixed models	4
Immunity			
Incidence rate of any upper respiratory infection	F914	Poisson regression	2
Incidence rate of flu-like illness	F914	Poisson regression	2
Incidence rate severe infections that lead to hospital admission over the 36-month period	F914	Poisson regression	2

Table of Secondary Endpoints and Suggested Method (continuation)

Bone/cartilage	Form	Method	Formula
Severity of knee pain in patients with symptomatic knee OA at baseline	KOOS	Linear mixed models	4
Rate of Knee buckling in patients with symptomatic knee OA at baseline	Knee bucking questionnaire	Logistic regression	3
Total number of joints with pain	McGill	Linear mixed models	4
NSAID use due to knee pain	F106/306	Logistic regression	3
Dental			
Decline in oral health	GOHAI	Linear mixed models	4
Tooth loss	F105/305	Linear mixed models	4
Gastro-intestinal			
Presence of GI symptoms	Rome III	Logistic regression	3
Number of any new gastro-intestinal symptoms	Rome III	Poisson regression	2
Glucose-metabolic			
Fasting blood concentrations of glucose and insulin	blood	Linear mixed models	4
Body composition and fat mass	DEXA	Linear mixed models	4
Kidney			
Decline in kidney function (creatinine levels and GFR)	blood	Linear mixed models	4
Global Health			
Quality of life	EUROQUOL	Linear mixed models	4
Frailty level (at least pre-frail vs. Robust)	SHARE	Logistic regression	3
Level of activities of daily living	PROMIS-HAQ	Linear mixed models	4
Incident mortality, incident permanent nursing home admissions, and the number of acute hospital admissions	F910	Logistic regression	3

7.2. Methods and formulas.

a) Cox proportional Hazards

Since follow-up times for the participants are likely to be different, we will compare time until the outcome among the treatment groups using **Cox survival models.** The proportional hazard assumption will be checked for each outcome and, if the assumption is satisfied, the Proportional Hazards Cox model will be used; otherwise we will use stratified Cox models. Both types of the Cox model will be defined in the same way:

$$h(t,\mathbf{X}) = h_0 t \times exp[\beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers] \quad (1)$$

X – incident secondary endpoint
t – time to event (non-vertebral fracture), death, drop-out or end of the study
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$\mathrm{Fall_i}$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85

b) Poisson Regression

For secondary endpoints using rates and counts, we assume a Poisson distribution for the number of infections, and will use a **Poisson regression** analysis to compare the number of infections for each intervention using indicators for treatment exposure, controlling for the stratification factors (age, gender, center, faller status) and BMI.

$$Log(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + +\varepsilon \quad (2)$$

Where:

Y – Rate of secondary endpoint
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$\mathrm{Fall_i}$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85
ε – error

c) Logistic Regression

To test the main effects of the study treatments on incidence rate of secondary endpoints, we will use **logistic regression.** We will estimate the odds ratio for each intervention controlling for the other two interventions, age, and gender, faller status and BMI

$$Logit(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + \beta_{14} \log(Time) \quad (3)$$

Where:

Y – incident non-vertebral fracture (first fracture only)
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85
Time – Follow up time since baseline

d) **Linear mixed models**

We will study the effects of the study treatments changes on continuous secondary endpoints by **linear mixed models**

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Age_i + \beta_5 Gender_i + \beta_6 Fall_i + \beta_7 BMI_i + \beta_{8-13} Centers_i + \gamma_{1-3} Time_{ij} + \gamma_{4-6}(Time_{ij} \times VD_i) + \gamma_{7-9}(Time_{ij} \times VD_i) + \gamma_{10-12}(Time_{ij} \times PE_i) + \varepsilon_{ij} \quad (4)$$

Where:

i – i-th patient (i = 1, 2, …, 2152);
j – j-th visit (j = 0 (baseline), 1 (12 months), 2 (24 months), 3 (36 months));
Y_{ij} – SPPB score for i-th patient at j-th visit;
$Time_{ij}$ – Follow$_1$ up time since baseline for i-th participant at j-th visit
VD_i – vitamin D treatment (2000 IU/day vs. placebo) for the i-th participant;
$\Omega 3_i$ – omega-3 fatty acids treatment (1 g/day vs. placebo) for the i-th participant;
PE_i – physical exercise program (strength vs. mobility) for the i-th participant;
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – linear spline with knot at age 85

DO-HEALTH statistical analysis plan and definitions of the variables

Version 1.~~6~~5.1 dated ~~03~~26.~~.01.~~02.20~~18~~20

Version 1.5 dated 03.01.2018

This document "statistical analysis plan Do-health" was developed by Dr. Patricia Chocano-Bedoya (Epidemiologist, Centre on Aging and Mobility, University Hospital Zurich) and Prof. Heike A. Bischoff-Ferrari (PI DO-HEALTH), under supervision and with final edits by Prof. E. John Orav (Prof. of Biostatistics, Harvard Medical School, and Head biostatistician of DO-HEALTH). The statistical methods final revision was on 11.12.2017 and final editorial changes were incorporated by 03.01.2018.

The version 1.5 is the final version implemented in DO-HEALTH data analyses of all protocol defined endpoints.

No analyses on the DO-HEALTH trial data set and with regards to the interventions were performed prior to the finalization of the DO-HEALTH analysis plan as outlined in this document.

The version 1.5.1 corrects the typo of the significance level for testing treatment group interactions described in section 5.1.1. Detailed explanation for this correction is included in appendix.

Prof. Dr. med. Heike A. Bischoff-Ferrari, Dr.PH
PI DO-HEALTH

Prof. Endel John Orav, PhD
Head biostatistician of DO-HEALTH

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Abbreviations and definitions

ACR	American College of Rheumatology
ALT	Alanine Aminotransferase
AST	Asparagine Aminotransferase
BMD	Bone Mineral Density
CoV	Coefficient of Variability
CRF	Case Report Form
CRP	C-reactive Protein
DHA	Docosahexaenoic Acid
DSMB	Data Safety and Monitoring Board
DXA or iDXA	Dual-energy X-ray Absorptiometry
EPA	Eicosapentaenoic Acid
FRAX®	WHO Fracture Risk Assessment Tool
GEE	Generalized Estimating Equation
GI	Gastro-intestinal
HDL	High Density Lipoprotein
HOOS	**Hip** injury and Osteoarthritis Outcome Score
Incidence	means that an individual has the event or not
ITT	Intention to Treat
KOOS	Knee injury and Osteoarthritis Outcome Score
LDH	Lactate Dehydrogenase
MAR	Missing at Random
MMSE	Mini Mental State Examination
OA	Osteoarthritis
PI	Principal Investigator
PUFA	Polyunsaturated Fatty Acid
SD	Standard Deviation
SPPB	Short Physical Performance Battery
TSH	Thyroid Stimulating Hormone

1. Study design and objectives

1.1. Study design

DO-HEALTH is a randomized, double-blind, placebo-controlled, 2×2×2 factorial, multi-center clinical trial. The trial is performed at 7 recruitment centers located in 5 countries: Switzerland (University of Zurich, Basel University Hospital, Geneva University Hospital), France (University of Toulouse Hospital Centre), Germany (Charité Berlin), Portugal (University of Coimbra), and Austria (Innsbruck Medical University).

1.2. Study objectives

Main objectives:

- To improve healthy ageing in European seniors
- To reduce healthcare costs via the implementation of effective and broadly applicable disease prevention interventions

Specific Objectives:

- To establish whether vitamin D, omega-3 fatty acids, and a simple home exercise program will prevent disease at older age
- To assess comparative effectiveness and cost-benefit of the interventions

2. Endpoints

2.1. Primary endpoints

Bone	Incidence of non-vertebral fractures
Muscle	Functional decline
Cardiovascular	Change in systolic and diastolic blood pressure
Brain	Cognitive decline
Immunity	Number of infections of any type

2.2. Secondary endpoints

Bone	Incidence of hip fractures
	Incidence of vertebral fractures
	Incidence of any fractures
	Bone mineral density (BMD)at the spine and hip
Muscle	Number of any low trauma falls
	Number of injurious falls
	Number of participants who fell
	Reaction time
	Grip strength
	Muscle mass in the upper and lower extremities
	Musculoskeletal pain
	Dual tasking gait speed

Cardiovascular	Incidence of hypertension over 36 month
Brain	Mental health decline
	Incidence of depression
	Dual tasking gait variability
Immunity	Number of any upper respiratory infections, flu-like illnesses, and severe infections that lead to hospital admission over the 36-month period
Bone/cartilage	Severity of knee pain in patients with symptomatic knee OA at baseline
	Rate of knee buckling in patients with symptomatic knee OA at baseline
	Total number of joints with pain
	NSAID use due to knee pain
Dental	Decline in oral health
	Tooth loss
Gastro-intestinal	Number of any new gastro-intestinal symptoms
Glucose-metabolic	Fasting blood concentration of glucose and insulin
	Body composition and fat mass
Kidney	Decline in kidney function
Global health	Quality of life
	Frailty level
	Level of activities of daily living
	Incident mortality, incident permanent nursing home admissions, and the number of acute hospital admissions

2.3. Biomarker endpoints

Bone	calcium, phosphate, 25(OH)D, intact PTH, urinary calcium/creatinine ratio (second spot urine), Beta-Crosslaps serum, P1NP
Cardiovascular	Troponin T, NT-proBNP, homocysteine, CK, cholesterol, HDL-cholesterol, triglycerides
Inflammation	CRP, IL6
Gastro-intestinal	AST, ALT, gGT, alkaline phosphatase, bilirubin
Glucose-metabolic	fasting glucose, insulin
Kidney	serum creatinine; calcium/creatinine ratio in second spot urine, serum urea, uric acid
Global Health	Ions: sodium, potassium, chloride, magnesium Proteins: total protein, albumin, ferritin, soluble transferrin receptor Hormones: TSH, fT4, fT3, cortisol Vitamins: folic acid, vitamin B12, 25(OH)D
Adherence to treatment	serum 25(OH)D, plasma PUFA concentrations (EPA, AA, DPA, DHA).
Bone (novel)	sclerostin
Muscle (novel)	myostatin
Inflammation (novel)	TNF-α, IL-10, IL-17, IL-22, IL-8*, IL-6*, IL-1β*
Cellular immunity (novel)	CD3, CD4, CD25, CD127

* not included in the current protocol

2.4. Exploratory endpoints

Bone	Incidence of repeat fractures
Muscle	Incident disability regarding activities of daily living
	Incident sarcopenia
	Incident frailty
	Level of physical activity
Cardiovascular	Incidence of major cardiovascular events
Brain	Incidence of dementia
Immunity	Incidence of cancer
	Number of implant infections after total hip or knee replacement due to fracture or osteoarthritis
	Number of gastro-intestinal infections
Bone/Cartilage-Arthritis	Incidence of symptomatic osteoarthritis
	Incident symptomatic hip osteoarthritis
	Incident symptomatic hand osteoarthritis
	Incident symptomatic knee
	Incident symptomatic knee, hip or hand osteoarthritis
	Severity of hand pain in those with prevalent symptomatic hip osteoarthritis
	Severity of hand pain in those with prevalent symptomatic hand OA

2.5. Ancillary Fracture healing study

Primary	Rate of clinical fracture healing
Secondary	Rate of functional fracture healing
Exploratory	Rate of radiological fracture healing

3. Screening

3.1. Screening Procedures

After a prospective participant had read the DO-HEALTH information and placed a call to the local DO-HEALTH telephone hotline, a trained study nurse conducted a pre-screening interview and scheduled a screening visit at the respective site for those who fulfilled the pre-screening criteria (see 'PreScreen' in 3.2)

At the screening visit, participants signed the informed consent form, completed the MMSE and had a blood test for calcium and creatinine levels. To ensure that also the minimal mobility function was met a screening walk and a chair test were performed with the prospective participant by the study nurse. Final eligibility of the prospective participant was assessed by the study MD and was depended on the answers to the inclusion /exclusion criteria, blood levels for serum calcium and creatinine, cognitive (MMSE ≥ 24) and physical function (screening walk and chair test) and the ability to swallow the study capsules.

3.2. Inclusion/Exclusion criteria

3.2.1. Inclusion criteria

- Age ≥ 70 years (PreScreen)
- Mini Mental State Examination Score of at least 24
- Living in the community (PreScreen)
- Sufficiently mobile to reach the study centre
- Able to walk 10 meters with or without a walking aid and getting in and out of a chair without help (PreScreen)
- Able to swallow study capsules
- Able and willing to participate, sign informed consent (including consent to analyze all samples until drop-out or withdrawal) and cooperate with study procedures

3.2.2. Exclusion criteria

- Consumption of more than 1000 IU vitamin D/day in the 3 months prior to enrollment, or unwillingness to reduce to 800 IU/d of vitamin D (current standard of care) for the duration of the trial (PreScreen)
    i. 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, may be enrolled after a 3-month wash-out period where the maximum daily intake is limited to 800 IU of vitamin D
    ii. Provision 2: an individual who consumed an average vitamin D dose higher than 2000 IU/day in the 3 months prior to enrollment, may be enrolled after a 6-month wash-out period where the maximum daily intake is limited to 800 IU of vitamin D
- Unwilling to limit calcium supplement dose to a maximum of 500 mg per day for the duration of the trial (PreScreen)
- Taking omega-3 fat supplements in the 3 months prior to recruitment and or unwilling to refrain from the use of omega-3 supplements for the duration of the trial (PreScreen)
- 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

- Current or recent (previous 4 months) participation in another clinical trial, or plans of such participation in the next 3 years (PreScreen)
- Presence of the following diagnosed health conditions in the last 5 years:
    i. History of cancer (except non-melanoma skin cancer)
    ii. Myocardial infarction, stroke, transient ischemic attack, angina pectoris, or coronary artery intervention
- Severe renal impairment (creatinine clearance ≤ 15 ml/min) or dialysis
- Hypercalcaemia (> 2.6 mmol/l)
- Hemiplegia or other severe gait impairment
- History of hypo- or primary hyperparathyroidism (PreScreen)
- History of granulomatous diseases (i.e. tubercolosis, sarcoidosis)
- Severe liver disease (PreScreen)
- Major visual or hearing impairment or other serious illness that would preclude participation (PreScreen)
- Living with a partner who is enrolled in DO-HEALTH (i.e. only one person per household can be enrolled) (PreScreen)
- Living in assisted living situation or in nursing home (PreScreen)
- Temporary exclusion: acute fracture in the last 6 weeks
- Epilepsy and/or use of anti-epileptic drugs
- Individuals who fell more than 3 times in the last month
- Osteodystrophia deformans (Paget's disease)
- For study centers in Germany only: persons who are institutionalized / in prison by court order (§40, Abs. 1, Art. 4, "Gesetz über den Verkehr mit Arzneimitteln").

4. Power

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

Power considerations for the primary endpoints.[*]

Endpoint	Minimum detectable difference[†]	Standard Deviation	Alpha	Power with no interact., N = 1806 (903 + 903)	Power with interaction, N = 902 (451 + 451)
Bone:[‡]					
Incidence rate of any non-vertebral fracture including hip fractures (proportion)	0.073[¶]	–	0.01	0.99	0.80
Muscle:[**]					
lower extremity function (mean SPPB score)	0.40	1.41	0.01	0.99	0.97
Cardiovascular:[**]					
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
Brain:[**]					
Cognitive function (mean MMSE score)	0.7	2.3	0.01	0.99	0.90
Immunity:[††]					
Incidence rate of any infection	0.32	s_1 = 1.46 s_2 = 1.35	0.01	0.99	0.90

[*] All assumptions about means and standard deviations of the parameters are based on the results of pilot trials to DO-HEALTH (9, 25, 26) and research of other authors(27-29).
[†] Minimum detectable difference was assumed as a minimum difference that is clinically significant.
[‡] Power calculations are based on the Z-test for difference between two proportions.
[¶] Estimated baseline prevalence of any non-vertebral fractures incidence rate / IR = 0.14.
[**] Power calculations are based on the two-sample t-test assuming equal variances.
[††] Power calculations are based on Poisson regression assuming non-equal variances.

5. General Principles for Data Analyses (adapted from protocol)

All analyses plans will be pre-defined and reviewed by the PI, the DO-HEALTH epidemiologist, and the head biostatistician (Prof. E. John Orav). All necessary procedures for the analyses plans and preparation of datasets will be coded in SAS statistical language and stored as electronic SAS files in the designated storage folders. An analysis log-book with a list of programs used per paper will be prepared for all analyses to be run for baseline comparisons and when the trial is finished.

The epidemiology team at the DO-HEALTH coordinating centre will conduct all statistical analyses using SAS v9.4 statistical software (Copyright© 2004by SAS Institute Inc., Cary, NC, USA) and supplemented by diagnostic techniques to assess the adequacy of statistical assumptions. Since the study assesses 5 primary endpoints, a Bonferroni adjustment for multiple comparisons will be applied in the statistical analysis so that $p < 0.01$ is required for statistical significance. In analysis for all other endpoints, a p-value of 0.05 (two-sided) will be considered a threshold for statistical significance.

Baseline analysis: The first analysis will compare baseline characteristics by randomized treatment assignment to ensure that balance was achieved by the randomization. Characteristics to be examined include known risk factors for the primary endpoints, including age, gender, BMI, smoking, alcohol use, physical activity, comorbidity, baseline blood pressure, baseline cognitive function (MMSE and MoCA score), baseline lower extremity function (SPPB score), prevalence of prior fall, and baseline 25(OH)D and omega-3 fatty acid blood levels. Baseline characteristics will be summarized by treatment group, where continuous variables will be presented as means and standard deviations and categorical variables will be presented as frequencies.

Analysis of treatment effects: In this 2×2×2 factorial design, the primary aim is to compare the main effects of **intention-to-treat** with vitamin D, omega-3 fats, and the home exercise program on the 5 primary endpoints as detailed in section 6.

5.1.1. Treatment interactions

We will assess the effect of one treatment while adjusting for the other treatments, also testing for a potential interaction effects between treatments in a two-stage process. First, we will compare a main effects model of vitamin D, omega-3 fatty acids and the home exercise program without interaction terms for the treatments. In the next step, we will explore the interaction between the treatment effects, using a statistical significance at $\alpha =$ ~~0.01~~0.10 to be included in the second model.

For the analysis of the primary endpoints, tests of treatment group interactions have been conducted using a significance level of 0.10 (See appendix for email communication between Dr. Patricia Chocano-Bedoya and Prof. E. John Orav dated August 29, 2019). There was a typo in version 1.5 dated 03.01.2018. In the following sentence "In the next step, we will explore the interaction between the treatment effects, using a Statistical significance at $\alpha = 0.01$ to be included in the second model", "$\alpha = 0.01$" should be corrected to be "$\alpha = 0.10$".

5.1.2.Confounding

The stratification variables for randomization included: recruitment center (7 centers), low trauma fall during previous 12 months prior to the randomization day (yes/no), gender, and age (70 – 84 and 85+).We

will assess the effect of one treatment while adjusting for the other treatments and for the stratification variables, as well as pre-defined potential confounders (BMI at baseline).

5.1.3. Missing data

- **Missing data on primary endpoints**

Missing data of <1% on any of the 5 primary outcomes will be considered negligible and missing completely at random (MCAR). Under MCAR assumptions, complete-case analysis (listwise deletion) reduces statistical power, but yields unbiased parameter estimates.

Missing data >1% on any of the 5 primary outcomes will be further inspected by a series of Little's MCAR X2 tests selecting on stratified variables. Non-significance suggests that the missing-data mechanism is ignorable and analyses will proceed with complete-case (listwise deletion) and available-case (pairwise deletion) datasets. MCAR significance will be addressed with two approaches to the treatment of missing data, which will enable a missing-model comparison with sensitivity implications on study outcomes. The first treatment of significant-missing data will be the carry-forward method (within-person / cold deck imputation). The second treatment of missing data will be multiple imputation (MI). MI is available in SAS via the 'PROC MI' and 'PROC MIANALYZE'.

This carry-forward and MI approaches will be used for continuous primary (function; blood pressure; and cognition) and secondary outcomes. For binary (non-vertebral fractures) and ordinal (number of infections) outcomes, the models will adjust for time-in-study and no carry-forward or imputation will be used.

6. Primary endpoints

6.1. Incidence of non-vertebral fractures

Background

All fracture events will be confirmed by X-ray reports or by medical records that describe an X-ray report of the fracture or mention the repair of the fracture. The endpoint was assessed every 3 month (4 visits, 9 phone calls) in all participants. Fracture classification cards will be standardized with the US VITAL trial (detailed fracture classification cards will be described in the fracture assessment manual to DO-HEALTH).

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce the incidence of non-vertebral fractures over the 36-month period (i.e., the outcome will be dichotomized so that subject will have either no fracture, or ≥1 fracture.):

Statistical analysis plan

- Main Analysis:

To test the main effects of the study treatments on incidence rate of non-vertebral fractures, we will use **logistic regression** to estimate the odds of having a fracture vs. not having a fracture. We will estimate the odds ratio for each intervention controlling for the other two interventions, age, and gender, faller status and BMI

$$Logit(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + \beta_{14} \log(Time\_in\_Study)$$

Where:

Y – incident non-vertebral fracture (first fracture only)
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age 85

Data dictionary

Variable Name	Definition		Frequencies/Descripts. (baseline)			Dataset/ Path	Questionnaire
		N (missing)	Range (IQR)	N (%)	Mean (SD)		
	MAIN VARIABLES:						
Fx_occurred	Fracture risk (proportion of at-risk population that experiences a fracture over study)	2157 (0)	0-1 (0-0)	254 (11.8)	NA	doh3.fractures_v10	Fracture Form F913
tot_Fx_Cnt	Incident cases (cumulative count of fracture events over study)	2157 (0)	0-4 (0-0)	0: 1903 (88.2) 1: 220 (10.2) 2: 29 (1.3) 3: 4 (0.2) 4: 1 (0.1)	.14 (.40)		Fracture Form F913
Days_To_First_Fx	Onset of first fracture event from study baseline	253 (1902)	13 - 1159 (330-839)	NA	570.19 (309.41)		Fracture Form F913
ftime	Follow-up time: Time to first fracture, until death, drop-out or till end of follow up	TBD					

SAS code for initial analysis / models

```
proc logistic data= doh3.fractures_v10 desc;
   class female (ref='1') faller
         berlin basel coimbra toulouse geneva innsbruck /param=ref  ;
   model Fx_occurred= tx_vitd tx_w3 tx_ex age female faller bmi
         berlin basel coimbra toulouse geneva innsbruck logtime;
run;
```

6.2. Functional decline (Primary endpoint)

Background

Functional decline will be assessed with the Short Physical Performance Battery (SPPB) (252), 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 (252). The SPPB is a brief performance-based test that includes walking speed, repeated chair stands, and a balance test. Its three components are each scored 0 to 4, with 4 indicating the highest level of performance, and are summed up to yield an overall score. The SPPB was assessed at all clinical visits: at baseline, 12, 24, and 36 months in all participants.

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce functional decline

Statistical analysis plan

- Main analyses:

We will use repeated measurement analyses and will estimate the mean change in SPPB for each intervention using indicators for treatment exposure and time, controlling for the other two interventions, stratification factors (age, and gender, faller status, center) and BMI. We will use the following formula**,** adjusting for unstructured correlation over time using generalized estimating equations:

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Age_i + \beta_5 Gender_i + \beta_6 Fall_i + \beta_7 BMI_i + \beta_{8-13} Centers_i + \gamma_{1-3} Time_{ij} + \gamma_{4-6}(Time_{ij} \times VD_i) + \gamma_{7-9}(Time_{ij} \times VD_i) + \gamma_{10-12}(Time_{ij} \times PE_i) + \varepsilon_{ij}$$

Where:

i – i-th patient (i = 1, 2, …, 2152);
j – j-th visit (j = 0 (baseline), 1 (12 months), 2 (24 months), 3 (36 months));
Y_{ij} – SPPB score for i-th patient at j-th visit;
$Time_{ij}$ – Follow$_1$ up time since baseline for i-th participant at j-th visit
VD_i – vitamin D treatment (2000 IU/day vs. placebo) for the i-th participant;
$\Omega 3_i$ – omega-3 fatty acids treatment (1 g/day vs. placebo) for the i-th participant;
PE_i – physical exercise program (strength vs. mobility) for the i-th participant;
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85

Data dictionary

Variable Name	Definition /Code	Frequencies/Descripts. (baseline)			Dataset/ Path	Questionnaire
		N (missing)	Range (IQR)	Mean (SD)		
MAIN VARIABLES:						
SPPB	Sum of Balance, Gait, Repeatchair scores Code: Sum (of **balance gait repeatchair**)	2150 (7)	3-12 (10-12)	10.87 (1.47)	Doh3.subjectsppb	F105
dSPPB	Change of SPPB over time (T1 to T3 minus baseline)	TBD				
SPPB_bl	Baseline SPPB	2150 (7)	3-12 (10-12)	10.87 (1.47)		
DERIVED FROM:						

balance	Measures ability to so side-by-site stands, semi-tandem stands and tandem stands. If a participant completed the TandemStand, they get 4 points
	
	Code:
	`if tandemStand10sec eq 1 then balance = 4;`
	`else if tandemstandtime ge 3 then balance = 3; *Tandem Stand for 3-9.99 seconds;`
	`else if semitandemStand10sec eq 1 then balance = 2; *Semitandemstand for 10 sec;`
	`else if sideBySideStand10sec eq 1 then balance = 1;`
	`else if tandemStand10sec eq . and tandemstandtime eq . and semitandemstand10sec eq . and sidebysidestand10sec eq . then balance=.;`
	`else balance=0;`
		2150 (7)	0-4 (4-4)	3.80 (0.53)	Doh3	F105

gait	Measures the time required to walk 4 meters at a normal pace (use the best of 2 times)
	
	Code:
	`gait=.;`
	`if mingait=. then gait=0;`
	`else if mingait lt 4.82 then gait=4;`
	`else if mingait lt 6.2 then gait=3;`
	`else if mingait lt 8.7 then gait=2;`
	`else gait=1;`
		2157 (0)	0-4 (4-4)	3.88 (0.43)	Doh3	F105

repeatchair	Measures the time required to perform five rises from a chair to an upright position as fast as possible without the use of arms. Code: `if repeatedchairtesttime =. then repeatchair=0; else if repeatedchairtesttime le 11.19 then repeatchair=4; else if repeatedchairtesttime le 13.69 then repeatchair=3; else if repeatedchairtesttime le 16.69 then repeatchair=2; else repeatchair=1;`	2157 (0)	0-4 (3-4)	3.8 (1.09)	Doh3	F105
ADDITIONAL VARIABLES FROM PHYSICAL TESTS:						
mingait	Best of two attempts Code: `min (of gaitspeedtestone gaitspeedtesttwo)`	2152 (5)	2.05-14.34 (3.03-3.98)	3.62 (.96)	Doh3	F105
meangait	Mean of two attempts Code: `mean (of gaitspeedtestone gaitspeedtesttwo)`	2152 (5)	2.08-17.15 (3.14-4.15)	3.78 (1.03)	Doh3	F105
gaitspeed	Mean time required to walk 1 meter Code: `gaitspeed=4/meangait;`	2152 (5)	.23-1.92 (.97-1.27)	1.12 (.23)	Doh3	F105
maxR	Maximum of grip in right hand Code: max (of gripR1 gripR2 gripR3)	2152 (5)	4-135 (48-70)	59.99 (18.44)	Doh3	F105
maxL	Maximum of grip in left hand Code: max (of gripL1 gripL2 gripL3)	2146 (11)	0-120 (46-70)	57.65 (18.71)	Doh3	F105
grip	Maximum dominant hand (R/L) Code: `if dominanthand='left' then grip=maxL; else grip=maxR;`	2152 (5)	4-135 (48-70)	60.05 (18.50)	Doh3	F105

SAS code for initial analysis / models

```
proc genmod data=data;
   class subjectid tx_vitD tx_w3 tx_ex numvisit female faller
         berlin basel coimbra toulouse geneva innsbruck;
   model SPPB = tx_vitD|numvisit tx_w3|numvisit  tx_ex|numvisit
                age female faller bmi
                berlin basel coimbra toulouse geneva innsbruck
                 /type3 residual;
   repeated subject = subjectid / type=un;
lsmeans tx_vitD *numvisit/cl pdiff;
lsmeans tx_w3 *numvisit/cl pdiff;
lsmeans tx_ex *numvisit/cl pdiff;
run;
```

6.3. Systolic and diastolic blood pressure changes (primary endpoint)

Background

Systolic and diastolic blood pressure changes will be assessed at baseline, 12, 24, and 36 months. Blood pressure will be measured after 5-minute rest in a seated position following a standardized protocol validated in a DO-HEALTH pilot trials.

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce systolic and diastolic blood pressure over 36 months.

Statistical analysis plan

- Main analyses:

We will study the effects of the study treatments on systolic/diastolic blood pressure by **linear mixed models**, as in 5.3

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Age_i + \beta_5 Gender_i + \beta_6 Fall_i + \beta_7 BMI_i + \beta_{8-13} Centers_i + \gamma_1 (Time_{ij} \times VD_i) + \gamma_2 (Time_{ij} \times PE_i) + \gamma_3 (Time_{ij} \times \Omega 3_i) + \varepsilon_{ij}$$

Where:

i – i-th patient (i = 1, 2, …, 2152);
j – j-th visit (j = 1, 2, 3);
Y_{ij} – Change in systolic/diastolic blood pressure for i-th patient at j-th visit;
$Time_{ij}$ – Follow$_1$ up time since baseline for i-th participant at j-th visit;
VD_i – vitamin D treatment (2000 IU/day vs. placebo) for the i-th participant;
$\Omega 3_i$ – omega-3 fatty acids treatment (1 g/day vs. placebo) for the i-th participant;
PE_i – physical exercise program (strength vs. mobility) for the i-th participant;
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);

Data dictionary (Alex, Matt/ R: Patricia)

Variable Name	Definition /Code	Frequencies/Descripts. (baseline)			Dataset/ Path	Questionnaire
		N (missing)	Range (IQR)	Mean (SD)		
MAIN VARIABLES:						
Syst_bl	Average of second and third measurements from predominant arm.	2145 (12)	94-217 (130-155)	143.46 (18.37)	dd.hypertension	F108
Diast_bl	Average of second and third measurements from predominant arm.	2145 (12)	47-113 (69-82)	75.88 (10.96)	dd.hypertension	F108
dSyst	Change in systolic blood pressure over time	TBD				
dDiast	Change in diastolic blood pressure over time	TBD				
ADDITIONAL VARIABLES:						
baselineBp_Arm_left	Predominant arm retained for averaging (measurement #s 2 & 3) BP values.	1041 (0)	0-1	48.2%	dd.hypertension	F108
baselineBp_Arm_right	Predominant arm retained for averaging (measurement #s 5 & 6) BP values.	1120 (0)	0-1	51.9%	dd.hypertension	F108
bp	Recoded into ordinal categories, from 0-5 0=optimal (120/80), 1=normal (130/85), 2=hi-normal (140/90), 3=stage1 (160/100), 4=stage2 (180/110), 5=stage3 (>180/>110)	2145 (12) 173 311 433 839 306 83	0-5 (2-3)	2.49 (1.24) 9.1% 14.4% 20.1% 38.9% 14.2% 3.8%	dd.hypertension	F108

SAS code for initial analysis / models

Similar code as for changes in SPPB:

```
proc mixed data=data;
   class subjectid tx_vitD tx_w3 tx_ex numvisit female faller
         berlin basel coimbra toulouse geneva innsbruck;
   model dSyst = tx_vitD|numvisit tx_w3|numvisit  tx_ex|numvisit
                 age female faller bmi
                 berlin basel coimbra toulouse geneva innsbruck /*oth cov*/
                 /solution residual;
   repeated /subject = subjectid type=un;
lsmeans tx_vitD *numvisit/cl pdiff;
lsmeans tx_w3 *numvisit/cl pdiff;
lsmeans tx_ex *numvisit/cl pdiff;
run;
```

6.4.Cognitive decline

Background

Cognitive decline will be assessed using the Montreal Cognitive Assessment (MoCA) at each of the clinical visits: at baseline, 12, 24, and 36 months. The structure of MoCA is similar to that of the Mini Mental State Examination (MMSE) and was found to be more sensitive than MMSE with respect to mild cognitive impairment. In addition subjective cognitive decline will be assed using the subjective memory complains questionnaire at the 36 months visits in a subset of 802 participants at two recruitment centers (Zurich and Basel).

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will improve cognitive function over 36 months.

Statistical analysis plan

We will study the effects of the study treatments on systolic/diastolic blood pressure by **linear mixed models**, as in 5.3

Data dictionary

Variable Name	Definition /Code	Frequencies/Descripts. (baseline)			Datase t/Path	Questionnaire
		N (missing)	Range (IQR)	Mean (SD)		
MAIN VARIABLES:						
Moca_bl	Total sum of moca scores corrected by education	2152 (5)	10-30 (24-28)	25.7 (3.4)	Doh3.moca	Moca
dMoCA	Change in moca scores over time	TBD				
ADDITIONAL VARIABLES:						
mocasumscore	Total sum of midscores 1-10 without correction by education	2152 (5)	9-30 (23-28)	25.2 (3.5)	Doh3.moca	Moca
eduyears	Total education years				dodata.f102demographics	F102
newedupoints	*add one point for an individuals with 12 or less years of education ; if eduyears le 12 then newedupoint=1;				Doh3.moca	Moca
VARIABLES USED FOR SUM OF MOCA SCORES:						
		N (missing)	Range	Freq		
Mocamidscore1	if mocamidscore1 eq . then do; mocamidscore1=sum (of trailMaking copyshape clockcontour clocknumbers clockhands);	2151 (6)	0-5	0:26 1:76 2:150 3:337 4:562 5:1001	Doh3.moca	Moca

Mocamidscore2		2151 (6)	0-3	0:6 1:22 2:161 3:1962		
Mocamidscore3		2152 (5)	0-2	0:119 1:509 2:1524		
Mocamidscore4	`if mocamidscore4 eq . then do;` `mocamidscore4=tapas;`	2152 (5)	0-1	0:260 1:1892		
Substract7		2153 (4)	0-3	0:35 1:72 2:281 3:1765		
Mocamidscore6	`if mocamidscore6 eq . then do;` `mocamidscore6=sum (of repeatsentence1 repeatsentence2 );`	2153 (4)	0-2	0:143 1:440 2:1570		
Mocamidscore7	`if mocamidscore7 eq . then do;` `mocamidscore7=words1minute;`	2152 (5)	0-1	0:857 1:1295		
Mocamidscore8	`if mocamidscore8 eq . then do;` `mocamidscore8=sum (of comparison1 comparison2);`	2153 (4)	0-2	0:100 1:531 2:1522		
Mocamidscore9	`if mocamidscore9 eq . then do;` `mocamidscore9=sum (of word1_3 word2_3 word3_3 word4_3 word5_3);`	2152 (5)	0-5	0:192 1:167 2:356 3:467 4:465 5:505		
mocamidscore10	`if mocamidscore10 eq . then do;` `mocamidscore10=sum (of date month year day place city);`	2153 (4)	0-6	2:2 4:13 5:100 6:2038		

SAS code for initial analysis / models

Same code as in 5.3

```
proc mixed data=data;
   class subjectid tx_vitD tx_w3 tx_ex numvisit female faller
         berlin basel coimbra toulouse geneva innsbruck;
   model dMoCA = tx_vitD|numvisit tx_w3|numvisit  tx_ex|numvisit
                 age female faller bmi
                 berlin basel coimbra toulouse geneva innsbruck
                 /solution residual;
   repeated /subject = subjectid type=un;
lsmeans tx_vitD *numvisit/cl pdiff;
lsmeans tx_w3 *numvisit/cl pdiff;
lsmeans tx_ex *numvisit/cl pdiff;
run;
```

6.5. Rate of infections

Background

Occurrence of infections will be assessed every 3 months (4 clinical visits and 9 phone follow-ups). Upon each contact, the participants will be asked whether any infections with or without fever have occurred and whether and when a vaccination was performed (i.e. flu vaccination). In case a participant has experienced infections, a detailed infection questionnaire developed in two pilot trials to DO-HEALTH will be applied. The questionnaire collects information about symptoms, treatment, MD contact and medical assessments. Every case of infection classified as a serious adverse event and any case of infection in the last three months of a participant's follow-up time will be confirmed by medical records. However, all reported infections, confirmed or not, will be used for the primary outcome.

Objectives

To test whether vitamin D, omega-3 fatty acids, and a simple home exercise program will reduce the rate of infections over the 36-month follow-up period.

Statistical analysis plan

For the rate of incident infections, we assume a Poisson distribution for the number of infections, and will use a **Poisson regression** analysis to compare the number of infections for each intervention using indicators for treatment exposure, controlling for the stratification factors (age, gender, center, faller status) and BMI.

$$Log(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + log(Time) + \varepsilon$$

Where:

Y – Rate of incident infections
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
Age – Linear spline for age with a knot at age 85
ε – error

SAS code for initial analysis / models

```
proc genmod data=data;
    class subjectid tx_vitD tx_w3 tx_ex female faller
          berlin basel coimbra toulouse geneva innsbruck /param=ref;
    model infection_inc =  tx_vitD tx_w3 tx_ex age female faller bmi
     berlin basel coimbra toulouse geneva innsbruck
     /dist=poisson link=log offset=exp_time;
estimate "Treatment (Group 1 vs. Control)" Treatment 1 0/exp;
estimate "Treatment (Group 1 vs. Control)" Treatment 0 1/exp;
run;
```

7. Secondary endpoints

7.1. Table of Secondary Endpoints and Suggested Method

Bone	Form	Method	Formula
Time to first fracture	F913	Cox Proportional Hazards	1
Total number of fractures (Incidence Rate)	F913	Poisson regression	2
Incidence of hip fractures	F913		
Cumulative risk of hip fracture	F913	Logistic regression	3
Time to first hip fracture	F913	Cox Proportional Hazards	1
Incidence of vertebral fractures	F913		
Cumulative risk vertebral fracture	F913	Logistic regression	3
Time to first vertebral fracture	F913	Cox Proportional Hazards	1
Bone mineral density (BMD)at the spine and hip	DEXA	Linear mixed models	4
Muscle			
Falls	F912		
Number of any low trauma falls (Incidence Rate)		Poisson regression	2
Number of injurious falls (Incidence Rate)		Poisson regression	2
Number of participants who fell		Logistic regression	3
Reaction time	F108/308	Linear mixed models	4
Grip strength	F108/308	Linear mixed models	4
Muscle mass in the upper and lower extremities	DEXA	Linear mixed models	4
Pain	McGill		
Musculoskeletal pain (total score)		Linear mixed models	4
Presence of pain lower/upper extremities		Logistic regression	3
Dual tasking gait speed	F108/308	Linear mixed models	4
Cardiovascular			
Diastolic Blood Pressure	F105/305	Linear mixed models	4
Incidence of hypertension over 36 month	F105/305	Logistic regression	3
Brain			
Mental health	GDS		
Mental health decline (continuous GDS scores?)		Linear mixed models	4
Incidence of depression		Logistic regression	3
Dual tasking gait variability	F108/308	Linear mixed models	4
Immunity			
Incidence rate of any upper respiratory infection	F914	Poisson regression	2
Incidence rate of flu-like illness	F914	Poisson regression	2
Incidence rate severe infections that lead to hospital admission over the 36-month period	F914	Poisson regression	2

Table of Secondary Endpoints and Suggested Method (continuation)

Bone/cartilage	Form	Method	Formula
Severity of knee pain in patients with symptomatic knee OA at baseline	KOOS	Linear mixed models	4
Rate of Knee buckling in patients with symptomatic knee OA at baseline	Knee bucking questionnaire	Logistic regression	3
Total number of joints with pain	McGill	Linear mixed models	4
NSAID use due to knee pain	F106/306	Logistic regression	3
Dental			
Decline in oral health	GOHAI	Linear mixed models	4
Tooth loss	F105/305	Linear mixed models	4
Gastro-intestinal			
Presence of GI symptoms	Rome III	Logistic regression	3
Number of any new gastro-intestinal symptoms	Rome III	Poisson regression	2
Glucose-metabolic			
Fasting blood concentrations of glucose and insulin	blood	Linear mixed models	4
Body composition and fat mass	DEXA	Linear mixed models	4
Kidney			
Decline in kidney function (creatinine levels and GFR)	blood	Linear mixed models	4
Global Health			
Quality of life	EUROQUOL	Linear mixed models	4
Frailty level (at least pre-frail vs. Robust)	SHARE	Logistic regression	3
Level of activities of daily living	PROMIS-HAQ	Linear mixed models	4
Incident mortality, incident permanent nursing home admissions, and the number of acute hospital admissions	F910	Logistic regression	3

7.2. Methods and formulas.

a) Cox proportional Hazards

Since follow-up times for the participants are likely to be different, we will compare time until the outcome among the treatment groups using **Cox survival models.** The proportional hazard assumption will be checked for each outcome and, if the assumption is satisfied, the Proportional Hazards Cox model will be used; otherwise we will use stratified Cox models. Both types of the Cox model will be defined in the same way:

$$h(t,\mathbf{X}) = h_0 t \times exp[\beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers] \quad (1)$$

X – incident secondary endpoint
t – time to event (non-vertebral fracture), death, drop-out or end of the study
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$\mathrm{Fall_i}$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85

b) Poisson Regression

For secondary endpoints using rates and counts, we assume a Poisson distribution for the number of infections, and will use a **Poisson regression** analysis to compare the number of infections for each intervention using indicators for treatment exposure, controlling for the stratification factors (age, gender, center, faller status) and BMI.

$$Log(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + +\varepsilon \quad (2)$$

Where:

Y – Rate of secondary endpoint
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$\mathrm{Fall_i}$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85
ε – error

c) Logistic Regression

To test the main effects of the study treatments on incidence rate of secondary endpoints, we will use **logistic regression.** We will estimate the odds ratio for each intervention controlling for the other two interventions, age, and gender, faller status and BMI

$$Logit(Y) = \alpha + \beta_1 VD + \beta_2 \Omega 3 + \beta_3 PE + \beta_4 Age + \beta_5 Gender + \beta_6 Fall + \beta_7 BMI + \beta_{8-13} Centers + \beta_{14} \log(Time) \quad (3)$$

Where:

Y – incident non-vertebral fracture (first fracture only)
VD – vitamin D treatment (2000 IU/day vs. placebo);
Ω3 – omega-3 fatty acids treatment (1 g/day vs. placebo)
PE – physical exercise program (strength vs. mobility)
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – Linear spline for age with a knot at age85
Time – Follow up time since baseline

d) **Linear mixed models**

We will study the effects of the study treatments changes on continuous secondary endpoints by **linear mixed models**

$$Y_{ij} = \alpha_i + \beta_1 VD_i + \beta_2 \Omega 3_i + \beta_3 PE_i + \beta_4 Age_i + \beta_5 Gender_i + \beta_6 Fall_i + \beta_7 BMI_i + \beta_{8-13} Centers_i + \gamma_{1-3} Time_{ij} + \gamma_{4-6}(Time_{ij} \times VD_i) + \gamma_{7-9}(Time_{ij} \times VD_i) + \gamma_{10-12}(Time_{ij} \times PE_i) + \varepsilon_{ij} \quad (4)$$

Where:

i – i-th patient (i = 1, 2, …, 2152);
j – j-th visit (j = 0 (baseline), 1 (12 months), 2 (24 months), 3 (36 months));
Y_{ij} – SPPB score for i-th patient at j-th visit;
$Time_{ij}$ – Follow$_1$ up time since baseline for i-th participant at j-th visit
VD_i – vitamin D treatment (2000 IU/day vs. placebo) for the i-th participant;
$\Omega 3_i$ – omega-3 fatty acids treatment (1 g/day vs. placebo) for the i-th participant;
PE_i – physical exercise program (strength vs. mobility) for the i-th participant;
$Fall_i$ – i-th participant's history of falls 12 months prior to enrollment into DO-HEALTH (yes/no);
Age – linear spline with knot at age 85

Appendix

Email communication between Dr. Patricia Chocano-Bedoya and Prof. E. John Ora regarding the test of treatment group interactions.

Dear John,

We tested first the interactions of all treatments. In the first model tx_vitd*tx_w3*tx_phys was not significant but tx_w3*tx_phys were at 0.07. We removed the 3-way interaction term from the model, and tested again only the 2-way interactions. The interaction term tx_w3*tx_phys was still significant (p=0.08). Since the other interaction terms were not significant, we run the model again only with one interaction term. This is the process we have been following for all tests.

Best,

Patricia

Von: Orav, Endel John,Ph.D. <eorav@bwh.harvard.edu>
Gesendet: Donnerstag, 29. August 2019 23:23
An: Chocano Patricia <Patricia.Chocano@usz.ch>; Bischoff Heike <Heike.Bischoff@usz.ch>; de Godoi Rezende Costa Molino Caroline <Caroline.deGodoiRezendeCostaMolino@usz.ch>; 'sandrine-rival@do-health.eu' <sandrine-rival@do-health.eu>
Betreff: RE: codes gi symptoms

Dear Patricia,

The process you outline below (i.e., check for treatment interactions first; if any are significant at p<0.10 then go to the 8-arm analysis) is consistent with what we have done for the other outcomes, and I think it is still the appropriate approach. And your 8-arm analysis is correctly interpreted as showing no significant benefit of any of the 8 interventions.

What I would question is the model that you've used to test for treatment interactions. This model should be consistent across all outcomes. And, my memory is that previously the model included terms for all three possible treatment interactions. Your current model only allows for the interaction between omega-3 and exercise. If my memory is correct, please use the same model we have used previously and see if the interaction still comes out with a p-value less than 0.10. If so, we are stuck with no benefit from any intervention of combination. (I'm hoping that the previous model used main effects for all three interventions first, and then the three interactions [so that the interaction terms are each 1 degree of freedom].)

If there's still time before you leave, please send me the code and results from the expanded interaction model.

Thanks, John

From: Chocano Patricia <Patricia.Chocano@usz.ch>
Sent: Thursday, August 29, 2019 3:46 AM
To: Orav, Endel John,Ph.D. <eorav@bwh.harvard.edu>; Bischoff Heike <Heike.Bischoff@usz.ch>; de Godoi Rezende Costa Molino Caroline <Caroline.deGodoiRezendeCostaMolino@usz.ch>; 'sandrine-rival@do-health.eu' <sandrine-rival@do-health.eu>
Subject: WG: codes gi symptoms

External Email - Use Caution

Dear John,

We would like to check with you our analyses regarding the Gastrointestinal symptoms in DO-HEALTH. In the prior analyses (see table attached), when we did the 3-arm analysis, omega-3 had a significant effect reducing gastrointestinal symptoms. However, the interaction between omega-3 and exercise is significant at 0.08 so we changed to the 8-arm model. Based on this analysis, none of the interventions has a significant effect. I have attached here the output for the interaction test and the 8-arm results (starting page 7). Below you can find our code.

Thank you,

Patricia

```
title "test for interaction: omega-3 and SHEP";

proc genmod data=newtrsumrome;

        class tx_vitD(ref="0") tx_w3(ref="0") tx_phys(ref="0") /param=GLM;

        model sumNewGI=tx_phys*tx_w3 tx_vitD tx_w3 tx_phys
                                                age85a age      female fall_yes bmi
                                                berlin basel coimbra toulouse geneva innsbruck
                                                sumBLGI
        /dist=poisson link=log offset=logpyears type3;
*lsmeans  tx_vitD*tx_w3/diff ilink diff exp cl ;
*lsmeans  tx_vitD*tx_phys/diff ilink diff exp cl ;
lsmeans  tx_phys*tx_w3/diff ilink diff exp cl;
run;
```

```
/* Final model - with txgroup - significant  interaction between treatment */

title "any NEW Gastrointestinal symptoms  - 8 arm model";

proc genmod data=newtrsumrome;

        class txgroup (ref="Placebo") /param=GLM;

        model sumNewGI=txgroup

                                        age85a age female fall_yes bmi

                                        berlin basel coimbra toulouse geneva innsbruck

                                        sumBLGI

                /dist=poisson link=log offset=logpyears type3;

        lsmeans txgroup / diff ilink exp cl;

run; * no significant effects;
```