A Mediterranean-like dietary pattern with vitamin D_3 (10 µg/day) supplements reduced rate of bone loss in older Europeans with osteoporosis at baseline: results of a one year randomised controlled trial

Amy Jennings[1], Kevin D Cashman[2,3], Rachel Gillings[1], Aedin Cassidy[1], Jonathan Tang[1], William Fraser[1], Kirsten G Dowling[2], George LJ Hull[2], Agnes AM Berendsen[4], Lisette CPGM de Groot[4], Barbara Pietruszka[5], Elzbieta Wierzbicka[5], Rita Ostan[6], Alberto Bazzocchi[7], Giuseppe Battista[8], Elodie Caumon[9], Nathalie Meunier[9], Corinne Malpuech-Brugère[10], Claudio Franceschi[11], Aurelia Santoro[6,9], Susan J Fairweather-Tait[1]

Author Affiliations:

[1]Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK

[2]Cork Centre for Vitamin D and Nutrition Research, School of Food and Nutritional Sciences

[3]Department of Medicine, University College Cork, Cork, Ireland

[4]Division of Human Nutrition, Wageningen University & Research, The Netherlands

[5]Department of Human Nutrition, Warsaw University of Life Sciences (WULS-SGGW), Warsaw, Poland

[6]C.I.G. Interdepartmental Centre “L. Galvani”, Alma Mater Studiorum, University of Bologna, Bologna, Italy

[7]Diagnostic and Interventional Radiology, The “Rizzoli” Orthopaedic Institute, Bologna, Italy.

[8]Department of Experimental, Diagnostic and Speciality Medicine, Alma Mater Studorium, University of Bologna, Bologna, Italy

[9]CHU Clermont Ferrand, CRNH Auvergne, F-63000 Clermont-Ferrand, France

[10]Université Clermont Auvergne, INRA, UNH, Unité de Nutrition Humaine, CRNH Auvergne, F-63000 Clermont-Ferrand, France

[11]Institute of Neurological Sciences (IRCCS), Bologna, Italy

Authors' last names for PubMed: Jennings, Cashman, Gillings, Cassidy, Tang, Fraser, Dowling, Hull, Berendsen, de Groot, Pietruszka, Wierzbicka, Ostan, Bazzocchi, Battista, Caumon, Meunier, Malpuech-Brugère, Franceschi, Santoro, Fairweather-Tait

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Corresponding Author: Susan Fairweather-Tait

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Abbreviations list: NU-AGE, new dietary strategies addressing the specific needs of the elderly population for healthy ageing in Europe; MD, Mediterranean diet; BMD, bone mineral density; BMI, body mass index; DXA, dual energy X-ray absorptiometry; fPYD, free pyridinoline; fDPD, free deoxypyridinoline; LC-MS/MS, liquid chromatography-mass spectrometry; 25(OH)D, 25-hydroxyvitamin D; PTH, parathyroid hormone.

Clinical Trial Registry number and website: The NU-AGE trial was registered at clinicialtrials.gov as NCT01754012.

Abstract

BACKGROUND: The Mediterranean diet (MD) is widely recommended for the prevention of chronic disease, but evidence for a beneficial effect on bone health is lacking.

OBJECTIVE: To examine the effect of a Mediterranean-like dietary pattern (NU-AGE diet) on indices of inflammation with a number of secondary endpoints, including BMD and biomarkers of bone and collagen degradation in a 1-y multi-center randomised controlled trial (RCT) (NU-AGE) in elderly Europeans.

DESIGN: A RCT was undertaken across 5 European centers. Subjects in the intervention group consumed the NU-AGE diet for 1-y by receiving individually tailored dietary advice, coupled with supplies of foods such as wholegrain pasta, olive oil and a vitamin D_3 supplement (10 µg/day). Participants in the control group were provided with leaflets on healthy eating available in their country.

RESULTS: 1294 participants (mean age 70.9 ± 4.0 y, 44% male) were recruited to the study and 1142 completed the 1-y trial. The Mediterranean-like dietary pattern had no effect on BMD (site specific or whole body); including compliance to the intervention in the statistical model did not change the findings. There was also no effect of the intervention on the urinary biomarkers, free pyridinoline or free deoxypyridinoline. Serum 25(OH)D significantly increased and PTH decreased ($p<0.001$) in the MD compared with the control group. Sub-group analysis of individuals with osteoporosis at baseline (site specific BMD T-score ≤ -2.5 SD) showed that the MD attenuated the expected decline in femoral neck BMD (n=24 MD group, n=30 control group, $p = 0.04$) but had no effect on lumbar spine or whole body BMD.

CONCLUSIONS: A 1-y intervention of the Mediterranean-like diet together with vitamin D_3 supplements (10 μg/day) reduced the rate of loss of bone at the femoral neck in individuals with osteoporosis but had no effect on those with BMD in the normal range.

The NU-AGE trial is registered at clinicialtrials.gov as NCT01754012.

INTRODUCTION

A Mediterranean dietary pattern (MD), widely recommended for the prevention of chronic disease, is characterized by a high intake of fruits, vegetables, nuts, unrefined cereals and olive oil, a moderately high intake of fish, a low-to-moderate intake of dairy products, a low intake of meat, and a moderate intake of alcohol (1, 2). Data from prospective cohort studies show that greater adherence to a MD is associated with a significant improvement in health status, including reduced total mortality (2) and reduced incidence of cardiovascular disease, cancer, Parkinson's and Alzheimer's disease (3). Randomised controlled trials confirm that the MD may protect against vascular disease, although the quantity and quality of evidence available is limited and highly variable (4).

There are relatively few studies examining the association between a MD and bone health (bone mineral density and/or fracture incidence) and the available data are conflicting (5). A review of population-based studies, which focussed on fracture as an outcome (6), suggested that one of the modifiable risk factors for bone health is adherence to a MD. This conclusion was based on post-hoc analysis of longitudinal data from 93,676 women aged 50-79 y at the start of the Women's Health Initiative study which reported that higher adherence to a MD was associated with a lower risk for hip fractures (7). Data from randomized controlled trials investigating the effect of the MD on measures of bone health are sparse due to the difficulties of undertaking a dietary intervention that is long enough (i.e. minimum one year's duration) to be able to detect changes in BMD.

The aim of the present study was to examine the effect of the MD on BMD and biomarkers of bone and collagen degradation, as pre-specified secondary outcome measures, in a 1-y

multi-center randomized controlled trial (NU-AGE) in elderly Europeans. The trial was designed to assess the effects of consuming a Mediterranean-like dietary pattern for 1-y on markers of inflammation as the primary outcome and a series of secondary health-related outcomes, which include BMD and biomarkers of bone and collagen degradation. The Mediterranean-like dietary pattern was tailored individually to complement habitual dietary patterns to maximise compliance.

STUDY DESIGN AND METHODS

The NU-AGE trial was conducted in five European centres (Bologna in Italy, Norwich in the United Kingdom, Wageningen in the Netherlands, Warsaw in Poland and Clermont Ferrand in France). A detailed description of the European Commission-funded NU-AGE project has been reported elsewhere (8).

Ethics approval

Local ethical approval was provided by the Independent Ethics Committee of the Sant'Orsola-Malpighi Hospital Bologna (Italy), the National Research Ethics Committee – East of England (UK), the Wageningen University Medical Ethics Committee (Netherlands), the Bioethics Committee of the Polish National Food and Nutrition Institute (Poland) and South-East 6 Person Protection Committee (France). All study procedures were in accordance with the ethical standards of the Helsinki Declaration. All participants gave informed consent before participating. The trial was registered at clinicaltrials.gov (NCT01754012).

Participants

Recruitment and selection criteria have been reported previously (9). Briefly, 1294 participants aged 65–79 y were recruited through local advertisements, media publicity, and general practitioner surgeries between April 2012 and January 2014 at the five recruitment centres. Study participants were free living and responsible for their own dietary choices. Ineligibility criteria included any clinically diagnosed chronic disease, use of corticosteroids or insulin medications, recent use of antibiotics or vaccinations, recent change in habitual medication, presence of food allergy or intolerance necessitating a special diet, presence of frailty according to the Fried criteria (10) or malnutrition (defined as BMI<18.5 kg/m^2 or >10% weight loss in the previous six months). Participants were randomly allocated to the intervention or control group (1:1 allocation ratio) after stratification by gender, age, frailty status (pre-frail or non-frail) and BMI. Randomization was performed by entering the described variables of a subject into a computer program that automatically allocates and generates a unique ID-code. Participants were informed about their group after randomization. Technicians performing laboratory analysis were blinded to the group assignment, but researchers carrying out BMD measurements were not blinded because of practical impossibilities, including the fact that the participants themselves knew which group they were in and were in a position to discuss this with researchers whilst undergoing measurements.

Dietary intervention

Participants randomised to the intervention group received individually tailored standardised dietary advice in order to meet the study dietary requirements, as described previously (9). The NU-AGE food based dietary guidelines were based on nutrient reference values and food-based dietary recommendations for older adults from each of the five countries where the intervention took place, the modified MyPyramid for Older Adults, and nutrient requirements from the European Commission and the Institute of Medicine (9). The individually tailored dietary advice, either given face-to-face or by telephone by a trained dietician or research nutritionist, was administered nine times during the year and supported by mail or e-mail. To aid compliance participants in the intervention group received commercially available foods to help them meet the dietary guidelines including wholegrain pasta, olive oil, low-fat low-salt cheese, and high-MUFA and high-PUFA margarine in all centres and frozen vegetable soup (in Italy only) and vitamin D_3 supplements. Participants completed 3-day food diaries and returned unused vitamin D_3 supplements at months four and eight to evaluate follow-up adherence and use of the provided foods. Participants randomised to the control group were asked to continue with their usual diet for the year and only received a generally available leaflet with national dietary guidance.

Compliance to the study protocol in both the intervention and control groups was evaluated with seven-day food diaries at the start and end of the one-year intervention. A scoring system was developed to measure adherence to the diet; sixteen dietary components were included, 12 for which the highest intakes were ideal (fruits, vegetables, legumes, low-fat dairyand cheese, fish, lean meat and poultry, nuts, eggs, olive oil, fluids and vitamin D

supplements), two for which moderate intake was ideal (wholegrains and alcohol) and two for which low intakes were ideal (salt and sweets). Each component was scored proportionally from zero to 10 and contributed equally to the final score, which ranged from 0 to 160, with a higher score representing better adherence to the diet. High compliers were defined as participants whose change in the NU-AGE Index was ranked in the top two quintiles and low compliers were those in the lowest two quintiles.

Outcome assessment

At baseline and after 1-y, trained nurses or researchers measured whole body BMD with the use of DXA according to standard protocols and training (Hologic Discovery Wi , Hologic, Bedford, MA (UK); Lunar iDXA, GE Health Care Madison, WI, USA, enCORE™ 2011 software version 13.6 (Bologna, Italy); Discovery QDR®, Hologic Inc., USA, software version 3, (Clermont-Ferrand, France); Lunar Prodigy, GE Health Care, Madison, WI, USA, enCORE™ 2011 software version 13.6 (Wageningen, the Netherlands and Warsaw, Poland. Additionally, at three of the intervention sites (Italy, UK and Poland) BMD was assessed at predefined anatomical regions, including the lumbar spine (L1 to L4) and proximal femur (including total hip and femoral neck BMD). Osteoporosis was defined as a T-score of ≤-2.5 SD below peak bone mass (11).

Measurements of urine fPYD and fDPD

Free pyridinium crosslinks in urine were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS), as described elsewhere (12). In brief, the LC-MS/MS method

quantified free pyridinoline (fPYD) and free deoxypyridinoline (fDPD) simultaneously from a single sample analysis. fPYD and fDPD were calibrated using commercial standards (Immundiagnostik, Bensheim, Germany), and acetylated pyridinoline as internal standard. Prior to LC-MS/MS analysis, a solid phase extraction procedure was carried out on urine samples pre-treated with hydrochloric acid. The acidified samples were extracted using cellulose packed columns and eluted with 0.2% heptafluoro-butyric acid (HFBA) in water. The inter- and intra-assay coefficient of variation (CV) were ≤9.9% between the assay working range of 2-200 nmol/L. fPYD and fDPD results obtained from LC-MS/MS analysis were adjusted against urine creatinine measurements, which was performed on the COBAS® C501 analyser (Roche, Burgess Hill, UK). The inter- and intra-assay CV was ≤3.1% across the assay working range (375-55000 µmol/L).

Measurement of serum 25-dihyroxyvitamin D and parathyroid hormone

Concentrations of total 25-hydroxyvitamin D (25(OH)D) [i.e. 25(OH)D_2 plus 25(OH)D_3] in all serum samples were measured at the laboratory of the Cork Centre for Vitamin D and Nutrition Research using a slightly modified version of the LC-MS/MS method that has been described in detail elsewhere (13) and is certified by the Centers for Disease Control and Prevention's (CDC) Vitamin D Standardization Certification Program (14). The modifications were effected so as to reduce the total run time per sample from 10 mins in our existing method to 7 mins in the current method thereby increasing our efficiency of analysis of the sample loads (see **Supplemental Table 1** for details of gradient and multiple reaction monitoring (MRM) parameters). The 3-epimer of 25-hydroxyvitamin D_3 was chromatographically resolved from 25(OH)D_3, and the isotopically labelled d_3-3-epi-

177 25(OH)D_3 was used as an internal standard to verify retention time and separation of 3-epi-
178 25(OH)D_3 and 25(OH)D_3 in each sample run. The mean intra-and inter-assay CVs of the
179 methods were 3.9% and 6.5%, respectively, for 25(OH)D_3 (using low, medium and high
180 concentrations of 33.5, 49.2 and 86.2 nmol/L, respectively). The mean intra-assay and inter-
181 assay CVs of the method were 12% and 7.1%, respectively, for 25(OH)D_2 (using low, medium
182 and high concentrations of 1.10, 6.57 and 13.9 nmol/L, respectively).

183

184 Serum parathyroid hormone (PTH) concentrations were measured at the Cork Centre for
185 Vitamin D and Nutrition Research in all serum samples with the use of an ELISA (intact PTH;
186 MD Biosciences Inc.) Intra-assay and inter- assay CVs were 3.0% and 5.1%, respectively (at a
187 concentration of 47.7 and 52.6 pg/ml, respectively).

188

189 **Statistical analysis**

190 The power calculation for the estimation of the required sample size for this trial was based
191 on a change in CRP (as the primary outcome measure) of 0.6 mg/L (SD 4), which required a
192 sample size of 1000 participants (two-sided, 80% power and 0.05 alpha). We increased this
193 number to 1250 to account for an anticipated dropout rate of 20%. A previous study
194 examining the effect of a dietary intervention and consumption of fortified dairy products
195 for 12 months on spine BMD in postmenopausal women observed changes of -0.045 g/cm^2
196 in the control group, 0.008 g/cm^2 in the calcium supplemented group and 0.053 g/cm^2 in the
197 dietary intervention group (15). Based on these data we would need 36 participants (18 per
198 group) to observe an effect on spinal BMD (two-sided, 99% power and 0.05 alpha). This

199 indicated that we had sufficient osteoporotic participants (see **Table 2**) in our study to
200 conduct stratified analysis on the effect of MD on BMD.

201

202 The normality of the data for each variable was tested using Kolmogorov-Smirnov Test and
203 the Shapiro-Wilk Test. Baseline characteristics are presented as mean SD or n (%) for
204 categorical variables, and baseline between-group differences were assessed using
205 independent sample t-tests or χ^2 tests. The effect of the intervention on changes in BMD
206 and bone biomarkers was assessed using linear mixed-effect models with participant
207 included as random effect, time, treatment group, time x treatment group interaction, and
208 the explanatory variables study centre, age, sex, baseline BMI, baseline calcium intakes and
209 baseline 25(OH)D were included. Where we observed a significant time*treatment
210 interaction we also tested if there was a study centre effect by including a three way
211 time*treatment*study centre interaction term in the model. For each variable, values <3 or
212 >3 SDs from the mean were considered outliers and removed. As data were not normally
213 distributed, the models were fitted on a log-transformed scale. To account for multiple
214 testing we applied a Bonferroni correction, with eight tests per group (three BMD measures
215 and five biomarkers). We calculated the site-adjusted mean difference in intake of dietary
216 components associated with bone health using ANCOVA. Data were analysed using Stata
217 version 14 (Stata Corp., College Station, TX, USA).

218

219 **RESULTS**

220 Of the 1294 participants recruited to the NU-AGE study n=1142 completed (11.7% drop out
221 rate) **(Supplemental Figure 1)**. Of these completers, n=562 in the control group and n=555

in the intervention group had whole-body DXA scans at baseline and follow-up and complete covariate data (97.8%). There were no significant differences in baseline characteristics between the two groups (**Table 1**). Osteopenia (defined as a lumbar spine T-score of <-1.5 SD below peak bone mass) was present in 37%, and osteoporosis (defined as a lumbar spine T-score of <-2.5 SD below peak bone mass) in 8% of participants at baseline.

After 1-y dietary intervention, there was no effect on BMD at any bone site (**Table 2**) or on the concentrations of urinary fDPD and fPYD or the fDPD: fPYD ratio (**Table 3**). There was a significant ($p<0.001$) time x treatment interaction in change in serum 25(OH)D over the 12 months (**Table 3**), where the mean concentration significantly increased in the intervention group (4.5 ng/mL; 95% CI 3.9, 5.1) but was unchanged in the control group (0.5 ng/mL; 95%CI -0.1, 1.0) ($p<0.01$). There was a significant ($p<0.001$) time x treatment interaction in change in serum PTH over the 12 months, where the mean concentration increased in the control group (3.9 pg/mL 95% CI 2.1, 5.6) but no significant change in the intervention group (-1.4 pg/mL 95% CI -3.1, 0.4) ($p<0.001$)(Table 3). There was no effect of study centre for serum 25(OH)D ($P=0.049$) or serum PTH ($P=0.755$).

When examining the sub-group of participants diagnosed with osteoporosis at baseline (n=54) there was a 0.9% difference between the groups in the change in femoral neck BMD (Table 2); BMD increased in the intervention group (0.008 g/cm^2 95% CI -0.001,0.018) and decreased in the controls (-0.009 g/cm^2 95%CI -0.018,-0.001) $P=0.04$). No effect of study centre was observed ($P=0.415$). The intervention had no effect on BMD measured at the lumbar spine or the whole body.

When examining changes in specific dietary components associated with bone health we observed a significant increase in intakes of olive oil, low fat dairy and calcium in the intervention group relative to controls (**Figure 2**).

DISCUSSION

In one of the first long-term intervention studies examining the effect of the MD on BMD, we have found that consuming a MD with 10 μg/day vitamin D_3 reduces the rate of femoral neck bone loss, but not total body or spinal BMD loss, in elderly people with osteoporosis. There were no beneficial changes in BMD in individuals with BMD in the normal range at baseline.

There is conflicting evidence from cross-sectional studies examining the association between the MD and BMD, and a lack of consistency regarding the BMD sites which are most affected. In Chinese adults aged 40-75y, higher scores for adherence to a MD, adapted for China, were positively and dose-dependently associated with higher BMDs at whole body, lumbar spine, total hip, femur neck, trochanter, intertrochanter, but not Ward's triangle area (2.41–3.96% higher, quintile 5 vs. quintile 1, all P-values < 0.001), after adjusting for age and gender (16). Higher intakes of whole grains, fruits, and nuts and a lower intake of red and processed meat were independently associated with higher levels of BMD at several bone sites, but vegetables, legumes, fish, monounsaturated fat/saturated fat ratio, and moderate alcohol consumption showed no independent associations with

BMD in this study. In Finnish women aged 65-71 y (17) lumbar spine, femoral neck and total BMD were not significantly different across the Baltic sea diet (BSD) or MD quartiles. Also, there were no significant associations of BSD and MD quartiles in the subgroup with osteoporosis. A study of 220 Greek women (mean age 48 ± 12 y) found no link between adherence to a MD and bone mass, but when Principal Components Analysis was used to differentiate 10 dietary patterns, a high consumption of fish and olive oil and low intake of red meat was positively associated with lumbar spine BMD (18). A study in 200 pre- and post-menopausal Spanish women showed that a higher habitual intake of fruits, vegetables and nuts was associated with higher total body BMD in post-menopausal women (19). A smaller study in 87 Italians aged 70.1 ± 4.9 y also showed that adherence to the MD was associated with a higher BMD (T score assessed by calcaneal quantitative ultrasound of the mid-calcaneus) with lowest adherence observed in the 15% osteoporotic subjects (20).

Although it is not possible to draw conclusions about cause and effect from cross-sectional data, an association between the MD, or some components of the diet, and bone health is reported in some studies. To our knowledge, no previous dietary intervention studies in elderly people have reported the effect of the MD on BMD, therefore the NU-AGE randomized controlled trial provides an important opportunity to clarify the relationship. Our multi-center trial results show that consuming a MD (together with vitamin D supplements) for a year had no effect on BMD (whole body or site specific) in older people, and even when we included the degree of compliance to the dietary change in the statistical model this did not change the findings (**Supplemental Table 2**). Sub-group analysis, however, showed a significant beneficial effect of the MD plus supplemental vitamin D_3 on

femoral neck (but not lumbar spine or whole body) BMD in subjects identified at baseline as having osteoporosis.

Phenolic compounds, as found in virgin olive oil, are suggested as one of the components of the MD responsible for the effect on bone; the proposed mechanism is modulation of the proliferative capacity and cell maturation of osteoblasts through increased alkaline phosphatase activity and deposition of calcium ions in the extracellular matrix (21). A randomized controlled trial reported that the consumption of a MD enriched with virgin olive oil for 2 y was associated with an increase in the bone biomarkers for bone formation, serum osteocalcin and procollagen 1 N-terminal propeptide (P1NP) concentrations, in elderly men (22), indicating that the MD increases bone formation rather than decreasing resorption. In our dietary intervention, we provided virgin olive oil to the intervention group to encourage subjects to consume more olive oil. Baseline olive oil intake was highest in Italy (9.6 ± 0.4 g/d) and lowest in France (2.1 ± 0.4 g/d) and although there were no significant differences in intake between the countries, the greatest changes in intakes were observed in France (6.0 g/d) and the lowest in Italy (1.0 ± 0.5 g/d). A reduction in sodium intake, as undertaken in the DASH diet study (23), may be one of the consequences of consuming a MD (with reduced processed meat intake, and increased intakes of fruits and vegetables), and this has been reported to have beneficial effects on bone health through a reduction in urinary calcium excretion (24). However, knowing the difficulties of accurately measuring sodium intake, we did not attempt to evaluate the effect of sodium intake on BMD. Similarly, for other dietary components that may impact on bone turnover, such as

312 vitamin K, we were unable to include them in our model due to the lack of reliable intake
313 data.

314

315 Although osteoporosis is assumed to be a risk factor for bone fracture, the evidence for a
316 protective effect of the MD on risk of fracture is conflicting. Post-hoc analysis of longitudinal
317 data (median follow-up time of 15.9 y) from the US Women's Health Initiative reported a
318 lower risk for hip (but not total) fractures with higher adherence to a Mediterranean diet in
319 women 50-79 y (7). Conversely, a smaller population-based study of shorter duration (8 y) in
320 France found that greater adherence to the MD was not associated with a decreased risk of
321 fractures in men and women aged 67 y on recruitment (25). In a prospective study in
322 European men and women (EPIC) with a mean age of 48.6 y, followed for a median of 9 y,
323 increased adherence to MD protected against hip fracture occurrence, particularly among
324 men (26). In the PREDIMED trial, an observational cohort study nested in the main trial,
325 found that a higher consumption of extra-virgin olive oil was associated with a lower risk of
326 osteoporosis related fractures in Mediterranean men and women, aged 55-80 y, at high
327 cardiovascular risk (21). As with the cross-sectional studies cited above, the effect of the MD
328 appears to be mediated through particular dietary components, such as virgin olive oil.

329

330 In our study, subjects in the intervention group were given vitamin D_3 supplements (10
331 µg/day), which significantly increased serum total 25(OH)D and reduced parathyroid
332 hormone concentrations in the whole intervention group (but not in the osteoporosis sub-
333 group) compared with the control group (Table 3). This may be a question of insufficient
334 power as the osteoporotic sub-group was small. In this combined intervention design it is

not possible to disentangle the relative influence of the MD and/or vitamin D on femoral neck BMD in osteoporotic subjects. However, it is likely that the daily dose of vitamin D_3 (10 µg) was too low to have a significant impact on bone loss. MacDonald et al (27) found that hip bone loss was attenuated when vitamin D_3 supplements of 1000 IU (25µg) were given daily for 1-y to postmenopausal women, but 400 IU (10 µg) had no effect. A systematic review of vitamin D supplementation and risk of fractures concluded that vitamin D supplements of 700-800 IU (17.5-20 µg) per day appears to reduce the risk of hip and any non-vertebral fractures in ambulatory or institutionalized elderly persons, but that a vitamin D dose of 400 IU (10 µg) per day is not sufficient for fracture prevention (28). It is also worth noting that the baseline serum 25(OH)D of the participants in this RCT at ~25 ng/ml, exceeded that suggested by the Institute of Medicine (i.e., 20 ng/mL) as covering the needs of nearly all individuals from a bone health perspective (29). The Endocrine Society, however, have suggested that to maximize the effect of vitamin D on calcium, bone, and muscle metabolism, the circulating 25(OH)D should be above 30 ng/ml (30). This latter threshold was only achieved in just under half of the intervention group in the present RCT (mean serum 25(OH)D at endpoint, 29 ng/ml).

The strength of this study is that it was a long-term (one year) RCT carried out in a relatively large number (over 1,000) of European men and women, designed to examine the effects of a Mediterranean-like diet on various health parameters, including bone health. One of the limitations is the relatively small size of the sub-group with osteoporosis, and the significant and interesting findings of differences in response between individuals with BMD in the normal range and those with osteoporosis needs to be verified in a future study.

In conclusion, our study showed that a 1-y intervention of the MD together with vitamin D_3 supplements (10µg/day) reduced the rate of loss of bone at the femoral neck in individuals with osteoporosis but had no effect on those with BMD in the normal range.

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Table 1: Baseline characteristics of the NU-AGE study participants according to intervention group

Characteristic	**Intervention diet**		**Control diet**		***P*=**
Sex, female	n=632	363 (57.4)	n=644	356 (55.3)	0.437
Age, y	n=632	70.7 ± 4.1	n=643	71.1 ± 3.9	0.046
Body mass index, kg/m^2	n=633	26.9 ± 4.2	n=643	26.7 ± 3.8	0.492
Calcium intakes, g/d	n=618	618 ± 912	n=622	895 ± 347	0.361
Lumbar spine BMD, g/cm^2	n=377	1.1 ± 0.2	n=379	1.1± 0.2	0.553
Femoral neck BMD, g/cm^2	n=379	0.8 ± 0.1	n=385	0.8 ± 0.1	0.328
Whole body BMD, g/cm^2	n=616	1.1 ± 0.1	n=621	1.1 ± 0.1	0.963
Osteoporosis, yes	n=377	27 (7.2)	n=380	37 (9.7)	0.370
Free Pyridinoline (fPYD), creatinine adjusted nmol/mmol	n=612	24.0 ± 7.3	n=620	24.3 ± 7.5	0.489
Free Deoxypyridinoline (fDPD), creatinine adjusted nmol/mmol	n=612	6.1 ± 1.9	n=619	6.2 ± 2.0	0.636
deoxypyridinoline to Pyridinoline ratio	n=612	0.3 ± 0.1	n=619	0.3 ± 0.1	0.180
Parathyroid hormone, pg/ml	n=483	44.3 ± 26.5	n=479	42.4 ± 23.6	0.223
25-hydroxyvitamin D, ng/ml	n=613	24.6 ± 9.1	n=619	24.8 ± 8.9	0.745

Values are mean ± SD or n= (%)

Table 2: Mean difference in bone mineral density after 1-y of follow-up in the intervention and control diet groups

	Intervention	**Control**	**P**
All participants			
Lumbar spine BMD, g/cm^2	n=338	n=325	
Baseline	1.060 (1.042,1.078)	1.045 (1.026,1.063)	
1-y	1.065 (1.047,1.084)	1.049 (1.030,1.067)	
Change	0.005 (0.002,0.009)	0.004 (0.000,0.007)	1.000
Femoral neck BMD, g/cm^2	n=342	n=326	
Baseline	0.820 (0.807,0.833)	0.809 (0.796,0.822)	
1-y	0.816 (0.804,0.829)	0.804 (0.791,0.817)	
Change	-0.004 (-0.006,-0.001)	-0.005 (-0.008,-0.002)	1.000
Whole body BMD, g/cm^2	n=551	n=557	
Baseline	1.099 (1.090,1.107)	1.092 (1.084,1.101)	
1-y	1.098 (1.089,1.106)	1.091 (1.082,1.099)	
Change	-0.001 (-0.003,0.000)	-0.002 (-0.003,0.000)	1.000
Osteoporosis subgroup[2]			
Lumbar spine BMD, g/cm^2	n=25	n=33	
Baseline	0.770 (0.743,0.797)	0.768 (0.745,0.791)	
1-y	0.782 (0.755,0.810)	0.779 (0.755,0.802)	
Change	0.012 (0.001,0.024)	0.011 (0.001,0.021)	1.000
Femoral neck BMD, g/cm^2	n=24	n=30	
Baseline	0.649 (0.624,0.673)	0.635 (0.614,0.656)	

1-y	0.657 (0.633,0.681)	0.625 (0.605,0.646)	
Change	0.008 (-0.001,0.018)	-0.009 (-0.018,-0.001)	0.040
Whole body BMD, g/cm^2	n=20	n=22	
Baseline	0.883 (0.867,0.899)	0.856 (0.841,0.870)	
1-y	0.885 (0.869,0.901)	0.860 (0.846,0.875)	
Change	0.002 (-0.004,0.008)	0.005 (-0.001,0.011)	1.000

Values are mean (95% CI) adjusted for study centre, age, sex, calcium intakes, use of vitamin D supplements, 25-hydroxyvitamin D levels and BMI (all measured at baseline). Participants were excluded from the analysis if outcome values were <3 or >3 SDs from the mean; [1]*P* = Bonferroni corrected p values for the time x treatment interaction. [2]Osteoporosis was defined as femoral neck BMD T-score <2.5 SD.

Table 3: Mean difference in bone biomarkers after 1-y of follow-up in the intervention and control diet groups

	Intervention	**Control**	**P[1]**
Free Pyridinoline, nmol/mmol	n=551	n=563	
Baseline	23.1 (22.6,23.7)	23.6 (23.0,24.1)	
1-y	23.6 (23.1,24.2)	23.6 (23.1,24.2)	
Change	0.5 (0.0,1.0)	0.1 (-0.4,0.6)	1.000
Free Deoxypyridinoline, nmol/mmol	n=551	n=560	
Baseline	5.88 (5.74,6.01)	6.02 (5.88,6.15)	
1-y	5.99 (5.85,6.12)	5.93 (5.80,6.07)	
Change	0.1 (0.0,0.2)	-0.1 (-0.2,0.0)	0.208
Free Deoxypyridinoline: free pyridinoline ratio	n=554	n=563	
Baseline	0.26 (0.25,0.26)	0.25 (0.25,0.26)	
1-y	0.25 (0.25,0.26)	0.25 (0.25,0.26)	
Change	0.00 (-0.01,0.00)	0.00 (-0.01,0.00)	1.000
Parathyroid hormone, pg/ml	n=468	n=467	
Baseline	40.7 (38.7,42.8)	38.5 (36.5,40.5)	
1-y	39.4 (37.3,41.4)	42.4 (40.2,44.5)	
Change	-1.4 (-3.1,0.4)	3.9 (2.1,5.6)	0.000
25-hydroxyvitamin D, ng/ml	n=548	n=562	
Baseline	24.6 (24.0,25.3)	24.1 (23.5,24.8)	
1-y	29.1 (28.4,29.8)	24.6 (24.0,25.2)	

Change	4.5 (3.9,5.1)	0.5 (-0.1,1.0)	0.000
Osteoporosis subgroup[2]			
Free Pyridinoline, nmol/mmol	n=24	n=30	
Baseline	24.0 (21.7,26.4)	23.9 (21.8,26.0)	
1-y	25.6 (23.2,28.0)	24.9 (22.8,27.0)	
Change	1.6 (-0.6,3.8)	1.0 (-1.1,3.1)	1.000
Free Deoxypyridinoline, nmol/mmol	n=24	n=30	
Baseline	6.69 (5.96,7.43)	6.30 (5.66,6.94)	
1-y	6.44 (5.72,7.16)	6.76 (6.10,7.43)	
Change	-0.3 (-0.9,0.4)	0.5 (-0.2,1.1)	1.000
Free Deoxypyridinoline: free pyridinoline ratio	n=24	n=30	
Baseline	0.28 (0.25,0.30)	0.26 (0.24,0.28)	
1-y	0.26 (0.23,0.28)	0.27 (0.25,0.29)	
Change	-0.02 (-0.05,0.00)	0.01 (-0.01,0.03)	0.192
Parathyroid hormone, pg/ml	n=19	n=24	
Baseline	44.4 (33.9,54.9)	43.0 (34.0,52.0)	
1-y	44.4 (34.2,54.5)	49.0 (39.4,58.5)	
Change	0.0 (-11.0,11.0)	6.0 (-2.4,14.4)	1.000
25-hydroxyvitamin D, ng/ml	n=23	n=29	
Baseline	23.9 (20.9,27.0)	24.3 (21.6,27.1)	
1-y	29.2 (25.8,40.2)	28.1 (25.2,31.1)	
Change	5.2 (1.7,8.8)	3.8 (0.7,6.9)	1.000

Values are mean (95% CI) adjusted for study centre, age, sex, calcium intakes, use of vitamin D supplements, 25-hydroxyvitamin D levels and BMI (all measured at baseline). Participants were excluded from the analysis if outcome values were <3 or >3 SDs from the mean;

P[1]= Bonferroni corrected p values for the time x treatment interaction. [2]Osteoporosis was defined as femoral neck BMD T-score <2.5 SD.

Figure legend

Figure 1. Mean difference in intake of dietary components associated with bone health after 1-y of follow-up in the intervention and control diet groups.