Mediterranean Diet and High Dietary Acid Load Associated with Mixed Nuts: Effect on Bone Metabolism in Elderly Subjects

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OBJECTIVES: To analyze the effect of differing diet on the acid load content on bone metabolism.

DESIGN: Multicentric, randomized, single-blind, parallel-group clinical trial.

SETTING: Outpatient clinics.

PARTICIPANTS: Two hundred thirty-eight elderly men and women aged 60 to 80 at high risk for cardiovascular disease were randomly assigned to three interventional groups: a recommended low-fat diet (control diet group), a Mediterranean diet supplemented with virgin olive oil, or a Mediterranean diet supplemented with mixed nuts.

MEASUREMENTS: Main outcomes were 12-month changes from baseline in bone formation and resorption markers and bone mass measured according to quantitative ultrasound scanning.

RESULTS: The baseline data on the anthropometric, bone densitometry, and biochemical variables did not differ between the three groups. Dietary potential renal acid load (PRAL) and daily net endogenous acid production (NEAP) at baseline did not differ between groups. After intervention, subjects allocated to the Mediterranean diet with mixed nuts had a significant increase of PRAL and NEAP. In comparison, subjects in the Mediterranean diet with nuts group had higher parathyroid hormone (PTH) levels (2.63, 95% confidence interval (CI) = –1.01–6.35, P = .02) and a nonsignificantly higher (0.31, 95% CI = –0.13–0.74, P = .14) urine free deoxypyridoxine/creatinine ratio, a marker of bone resorption, than the control group and the Mediterranean diet with virgin olive oil group.

CONCLUSION: A Mediterranean dietary pattern associated with a high dietary acid load derived from consumption of mixed nuts does not seem to have a much greater effect on bone metabolism biomarkers, with the exception of PTH levels, than a Mediterranean diet without mixed nuts or a control diet in elderly subjects. J Am Geriatr Soc 57:1789–1798, 2009.

Key words: dietary acid load; nuts; Mediterranean diet; bone metabolism; clinical trials

Osteoporosis represents a major healthcare problem in developed countries and is projected to increase with the aging of the population, due in part to adverse changes in lifestyle and diet.1 Traditionally, nutritional research on bone metabolism has focused on the beneficial effects of calcium and vitamin D.2,3 Therefore, in recent decades, acid–base homeostasis has gained an increasing role in the etiology of bone disorders. It is well accepted that alkaline bone mineral protects against acidosis, and several studies have reported greater excretion of calcium and markers of bone resorption during metabolic acidosis.4,5 Moreover, studies performed in vitro show that chronic acidosis-like metabolic conditions decrease osteoblastic action and increase osteoclast resorptive activity.6,7 Therefore, less expression and activity of osteoblast alkaline phosphatase has been observed in vitro after reducing pH in the medium.8,9 Accordingly, Western diets generating a large amount of acid have been reported to increase urinary calcium and C-telopeptide excretion,9 whereas a base-forming diet rich in fruit and vegetables or supplemented with organic salts of potassium improves calcium balance and rates of bone formation markers and reduces bone resorption.10–12

Daily net endogenous acid production (NEAP) and dietary potential renal acid load (PRAL) are established methods for estimating a diet’s acidosis and acid load. They provide an estimation of how far endogenous acid produc-
tion exceeds alkali production, taking into account the intestinal absorption rates of individual minerals and proteins. In this sense, a cross-sectional study in women reported lower estimates of energy-adjusted NEAP in subjects with greater spine and hip bone mineral density (BMD) and higher levels of deoxyypyridinoline excretion in higher quartiles of estimated NEAP. More recently, high PRAL has been associated with lower calcaneal broadband ultrasound attenuation (BUA) in women but not in men. Foods that contribute to a lower acid load and therefore to a lower PRAL and lower estimated NEAP values include fruits, vegetables, and red wine, all of which are common in a traditional Mediterranean dietary pattern. In contrast, tree nuts, which are frequently consumed in large quantities in the traditional Mediterranean diet, are responsible for providing an important acid load, which may adversely affect bone health. Thus, a Mediterranean diet should exert a beneficial effect in terms of bone metabolism, whereas the consumption of nuts should have a detrimental effect on bone health.

For these reasons, the purpose of the present study was to evaluate the effect of a traditional Mediterranean food pattern on bone metabolism in comparison to a low-fat dietary pattern in an elderly population. Whether a higher dietary acid load in the context of a Mediterranean-style diet enriched with added nuts or enriched with virgin olive oil could modulate bone turnover, leading to a greater risk of developing osteopenia and osteoporosis, was also evaluated.

METHODS

Participants and Recruitment

The present study was conducted on a group of 407 elderly subjects who were recruited for the Prevención con Dieta Mediterránea (PREDIMED) Study, a multicenter, randomized, single-blind, parallel-group clinical trial that aims to evaluate the effect of the Mediterranean diet on cardiovascular mortality. The trial protocol has been described elsewhere (http://www.predimed.org). Primary care physicians recruited subjects based on review of their clinical records and a screening visit. Physicians obtained a list of candidates from computer-based records of patients attending each participating center and reviewed their clinical records to exclude those not meeting eligibility criteria. Suitable candidates were invited over the telephone to attend a screening visit that included an interview with administration of a 26-item questionnaire to inquire about medical conditions and risk factors related to eligibility. Eligible subjects were community dwelling and attended the PREDIMED network center in Reus, Spain; men were 55 to 80 and women aged 60 to 80. All were free of cardiovascular disease (CVD) at baseline, and all met at least one of the following criteria: type 2 diabetes mellitus (previously diagnosed with type 2 diabetes mellitus or fasting plasma glucose concentration ≥125 mg/dL observed on two consecutive occasions) or three or more coronary heart disease risk factors (hypertension (blood pressure >140/90 mmHg or treatment with antihypertensive drugs), low-density lipoprotein cholesterol (LDL-C) ≥160 mg/dL (or treatment with hypolipidemic drugs), high-density lipoprotein cholesterol (HDL-C) ≥40 mg/dL in men or ≥50 mg/dL in women), body mass index (BMI) ≥25 kg/m², or family history of premature CVD). People were excluded from taking part in the study if they were smokers, had a BMI of 35 kg/m² or greater, had lost more than 5% of their body weight in the previous year, had a history of cardiovascular disease, used medication known to affect bone metabolism (corticosteroids, bisphosphonates, thiazides, vitamin or mineral supplements), had been treated with hormone replacement therapy, had any severe chronic illness, abused drugs or alcohol, had a history of allergy or intolerance to olive oil or nuts, or were predicted to be unlikely to change their dietary habits according to the Prochaska and DiClemente stages-of-change model. Written informed consent for all of the study procedures was obtained from each subject in advance. The ethical committee of the Sant Joan University Hospital (Reus, Spain) approved the study protocol.

Randomization and Intervention

After the screening visit, a random-number sequence was used to assign each participant to one of the three diet intervention groups. Subjects assigned to the control diet group were recommended orally and in writing to follow this type of diet according to specific guidelines issued by the American Heart Association, focusing in the reduction of all types of fat (from animal and vegetable sources). Those assigned to the two Mediterranean diet groups were given personalized dietary recommendations during a 30-minute session on the intake of specific foods, including the use of olive oil for cooking and dressing; increasing consumption of fruits, vegetables, and fish; or increasing the consumption of white meat instead of red or processed meat. Participants assigned to the Mediterranean diet groups were given free extra virgin olive (1.5 L for 3 months) oil or sachets of mixed nuts (1,350 g walnuts (15 g/d), 675 g hazelnuts (7.5 g/d), and 675 g almonds (7.5 g/d)), depending on the group they had been assigned to. To improve participants’ adherence to the diet, the dietician delivered a 1-hour group session with up to 20 participants, with separate sessions for each Mediterranean diet group, every 3 months.

Measurements

The baseline examination included administration of a 14-item questionnaire assessing degree of adherence to the traditional Mediterranean diet that is an extension of a previously validated questionnaire. Food consumption was determined using a previously validated semiquantitative 137-item food frequency questionnaire (FFQ), and energy and nutrient intake were calculated from Spanish food composition tables. Physical activity was evaluated using the validated Spanish version of the Minnesota Leisure Time Physical Activity Questionnaire. The dietary PRAL index was calculated using individual nutrients derived from the FFQ and the following equation:

\[
\text{PRAL (mEq/d)} = ([mg \text{P}^2/d \times 0.0366] + [g \text{protein/d} \times 0.4888])
- ([mg \text{K}^+/d \times 0.0205] + mg \text{Ca}^{2+}/d \times 0.0125)
+ mg \text{Mg}^{2+}/d \times 0.0263)
\]
Estimated NEAP was calculated using an algorithm previously developed:\textsuperscript{24}

\[
\text{NEAP (mEq/d)} = 54.5 \times (\text{g protein/mEq K}^+) - 10.2
\]

and using the Remer equation,\textsuperscript{25} taking into account the anthropometry-based estimation for organic acid excretion:

\[
\text{NEAP (mEq/d)} = \text{PRAL (mEq/d)} + \text{estimated organic acid (OAest)} \times (\text{mEq/d}),
\]

where OAest (mEq/d) = individual body surface area \( \times \) 41.173.

Individual body surface area was calculated according to the formula of DuBois and DuBois,\textsuperscript{26} as follows:

\[
\text{body surface area } (m^2) = \left( \frac{0.007184 - \text{height (cm)}^{0.725}}{- \text{weight (kg)}^{0.425}} \right)
\]

The subjects wore light clothing and no shoes while their body weight and height were measured and their BMI calculated. Waist circumference was measured midway between the lowest rib and the iliac crest. Blood pressure was measured in triplicate using a validated semiautomatic oscillometer with a 5-minute interval between each (Omron HEM-705CP, Hoofddorp, the Netherlands), following the procedures recommended by the European Hypertension Society.\textsuperscript{27}

BMD (g/cm\textsuperscript{2}), broadband ultrasound attenuation (BUA; dB/MHz), and speed of sound (m/s) were measured at least twice on each calcaneum using a quantitative ultrasound scanner (Sahara, Hologic, Barcelona, Spain). T- and Z-score values were calculated using the original Caucasian reference population of the Hologic device. The scanner was calibrated daily using a physical phantom.

Urine was collected every 24 hours in flasks protected from light, divided into aliquots, and frozen at \(-80\)\textdegree C for later analysis. A urine sample was also collected for measuring urinary pH. Blood samples were collected under fasting conditions, centrifuged, and frozen at \(-80\)\textdegree C within 2 hours of collection until assay.

An automatic analyzer was used in a routine laboratory test to measure blood glucose, total cholesterol, HDL-C, and triglycerides. In patients whose triglyceride levels were less than 400 mg/dL, LDL-C concentrations were estimated using the Friedewald formula.\textsuperscript{28}

Serum intact parathyroid hormone (PTH), alkaline phosphatase isoenzymes (oAlp) (intra- and interassay coefficients of variation \(<10\)\%), and calcium levels were measured using chemiluminescence, the agarose gel method, and molecular absorption spectrometry, respectively (Cerba Internacional, Barcelona, Spain). Serum levels of 25-OH-vitamin D and osteoprotegerin were assessed using commercial enzyme-linked immunosorbent assays (Immunodiagnostik, Bensheim, Germany); intra- and interassay coefficients of variation ranged from 10.7\% to 13.2\%.

Chemiluminescence detection was used to measure free deoxypyridinoline (fDPD) excretion in 24-hour urine samples (Cerba Internacional, Barcelona, Spain). Urinary creatinine and calcium excretion were measured using molecular absorption spectrometry (Cerba Internacional). Urinary excretion of fDPD and calcium were corrected for creatinine excretion measured in the same urine sample.

### Statistical Analyses

Sample size has been calculated with 90\% power, \( \alpha = 0.05 \) (two-sided), to detect significant differences in changes in bone biochemical markers based on results of a previous study.\textsuperscript{12} Fifty-three patients were required for each study group. Two hundred thirty-eight patients were randomized to compensate for those that declined follow-up or possible deaths. Repeated-measures analyses of variance were used to explore differences in variables between the three interventions and between pairs of interventions; Bonferroni post hoc corrections were applied to correct the multiple comparison–related increase in alpha error. The chi-square test was used for categorical variables. NEAP and PRAL were evaluated, unadjusted and adjusted for total energy intake, by saving the residuals of the regression, using the Westen method. Means and standard deviations are presented for quantitative variables and are expressed as within-group differences (average changes and 95\% confidence intervals (CIs)). Skewed variables were analyzed using nonparamet-

### Table 1. Anthropometric Data and Densitometric Values at Baseline

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Diet Group (n = 59; 29 men, 30 women)</th>
<th>Mediterranean Diet with Virgin Olive Oil Group (n = 73; 34 men, 39 women)</th>
<th>Mediterranean Diet with Mixed Nuts Group (n = 70; 41 men, 29 women)</th>
<th>( P )-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>67.8 ± 6.1</td>
<td>67.8 ± 6.5</td>
<td>68.4 ± 6.0</td>
<td>.80</td>
</tr>
<tr>
<td>Body mass index, kg/m\textsuperscript{2}</td>
<td>29.6 ± 3.2</td>
<td>29.2 ± 3.0</td>
<td>29.4 ± 3.3</td>
<td>.75</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>101.5 ± 7.8</td>
<td>100.3 ± 7.7</td>
<td>101.2 ± 8.2</td>
<td>.64</td>
</tr>
<tr>
<td>Bone mineral density, g/cm\textsuperscript{2}</td>
<td>0.57 ± 0.15</td>
<td>0.53 ± 0.13</td>
<td>0.52 ± 0.08</td>
<td>.15</td>
</tr>
<tr>
<td>T-score</td>
<td>-0.27 ± 1.31</td>
<td>-0.60 ± 1.15</td>
<td>-0.69 ± 0.73</td>
<td>.20</td>
</tr>
<tr>
<td>Z-score</td>
<td>0.72 ± 1.26</td>
<td>0.38 ± 1.18</td>
<td>0.28 ± 0.76</td>
<td>.17</td>
</tr>
<tr>
<td>Broadband ultrasound attenuation, dB/MHz</td>
<td>84.4 ± 21.5</td>
<td>77.4 ± 19.7</td>
<td>75.9 ± 12.5</td>
<td>.25</td>
</tr>
<tr>
<td>Speed of sound, m/s</td>
<td>1,557.8 ± 36.5</td>
<td>1,549.65 ± 31.5</td>
<td>1,546.5 ± 20.5</td>
<td>.21</td>
</tr>
</tbody>
</table>
Table 2. Biochemical Values at Baseline

<table>
<thead>
<tr>
<th>Biochemical Value</th>
<th>Control Diet Group (n = 59)</th>
<th>Mediterranean Diet with Virgin Olive Oil Group (n = 73)</th>
<th>Mediterranean Diet with Mixed Nuts Group (n = 70)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose level, mg/dL</td>
<td>122.3 ± 33.6</td>
<td>118.3 ± 33.6</td>
<td>122.0 ± 43.3</td>
<td>.79</td>
</tr>
<tr>
<td>Total cholesterol level, mg/dL</td>
<td>210.0 ± 44.1</td>
<td>218.9 ± 37.5</td>
<td>209.7 ± 37.4</td>
<td>.33</td>
</tr>
<tr>
<td>HDL cholesterol level, mg/dL</td>
<td>56.3 ± 12.7</td>
<td>57.8 ± 13.9</td>
<td>58.1 ± 14.9</td>
<td>.76</td>
</tr>
<tr>
<td>LDL cholesterol level, mg/dL</td>
<td>127.1 ± 37.3</td>
<td>134.3 ± 33.8</td>
<td>125.4 ± 34.1</td>
<td>.32</td>
</tr>
<tr>
<td>Triglyceride level, mg/dL</td>
<td>151.0 ± 92.4</td>
<td>133.7 ± 69.1</td>
<td>130.3 ± 67.2</td>
<td>.29</td>
</tr>
<tr>
<td>Serum calcium, mg/dL</td>
<td>9.5 ± 0.3</td>
<td>9.6 ± 0.4</td>
<td>9.6 ± 0.4</td>
<td>.81</td>
</tr>
<tr>
<td>Serum parathyroid hormone, pg/mL</td>
<td>55.6 ± 22.8</td>
<td>56.7 ± 21.1</td>
<td>54.8 ± 19.3</td>
<td>.87</td>
</tr>
<tr>
<td>Serum alkaline phosphate level, U/L</td>
<td>68.9 ± 21.5</td>
<td>74.9 ± 22.2</td>
<td>73.3 ± 19.8</td>
<td>.27</td>
</tr>
<tr>
<td>Serum bone-specific alkaline phosphatase, %</td>
<td>43.8 ± 13.5</td>
<td>41.2 ± 12.7</td>
<td>44.0 ± 13.7</td>
<td>.41</td>
</tr>
<tr>
<td>Osteoprotegerin, pmol/L</td>
<td>5.1 ± 1.7</td>
<td>4.6 ± 1.6</td>
<td>4.8 ± 2.1</td>
<td>.31</td>
</tr>
<tr>
<td>Serum 25-OH-vitamin D, nmol/L</td>
<td>30.3 ± 21.0</td>
<td>30.1 ± 28.8</td>
<td>37.7 ± 36.9</td>
<td>.35</td>
</tr>
<tr>
<td>Deoxypyridinoline:creatinine ratio, nmol/mmol</td>
<td>5.9 ± 1.8</td>
<td>5.6 ± 2.3</td>
<td>5.5 ± 1.6</td>
<td>.50</td>
</tr>
<tr>
<td>Urinary calcium:creatinine ratio, mg/mg</td>
<td>0.15 ± 0.09</td>
<td>0.16 ± 0.10</td>
<td>0.14 ± 0.09</td>
<td>.70</td>
</tr>
</tbody>
</table>

Results. Analyses were done with SPSS software (version 11.0; SPSS Institute, Inc., Cary, NC).

RESULTS

Of the 407 eligible subjects initially screened, 136 were excluded because they did not meet the specific inclusion criteria of the present study. Several patients (n = 69) were excluded from the final analysis for several reasons (Figure 1), 38 because they did not collect the urine samples correctly.

Subject Characteristics

Table 1 summarizes the baseline anthropometric and bone densitometric characteristics of the 202 volunteers included in the study. No differences in baseline BMI, body fat distribution, mean age, or sex distribution were observed between groups. They were also homogeneous in their BMD, BUA, and T- or Z-scores. The baseline data on glucose and lipid profile and bone biochemical markers did not differ between the three study groups (Table 2).

Food, Energy, and Nutrient Intake

Estimated leisure-time energy expenditure from physical activity was similar in the three groups at baseline and after 1 year (data not shown). No changes in medication were observed. Using the results of the 137-item validated FFQ, Table 3 shows food intake at baseline and changes observed 1 year after the trial. No significant differences were observed in relation to baseline food consumption between groups except for meat intake. As expected, the most relevant changes were a significant increase in nut consumption in subjects allocated to the Mediterranean diet with mixed nuts and a significant increase in virgin olive oil consumption (which in part substituted for refined olive oil) in subjects allocated to the Mediterranean diet with virgin olive oil after 1 year of intervention. Subjects on the Mediterranean diet with mixed nuts tended to have only a slightly lower increase in consumption of base-forming foods such as fruits, vegetables, and red wine than participants in the other groups but tended to have larger increases in their intake of acidogenic foods such as fish, walnuts, and almonds (P<.001). After 12 months, subjects assigned to the Mediterranean diet with virgin olive oil and the Mediterranean diet with mixed nuts groups increased their average 14-unit Mediterranean diet score by 1.17 and 2.10 units, respectively, whereas the control diet group remained almost unchanged at 0.94 units (P = .01 for the between-group comparison).

Energy and nutrient intake at baseline did not differ significantly between groups. Nor were significant differences observed between the three groups in baseline dietary PRAL or baseline estimated daily NEAP (Table 4). After the intervention, significant changes were observed in fat intake (as a percentage of total energy intake), total fat intake was greater in the Mediterranean diet with mixed nuts group than in the control diet group (Table 4). No significant changes were observed in intake of dietary calcium or vitamin D. Subjects on the Mediterranean diet with mixed nuts also had significantly greater energy intake from polyunsaturated fatty acids (PUFAs) than the other two groups (P<.05). PRAL and NEAP, expressed as absolute values,
increased in subjects on the Mediterranean diet with mixed nuts but decreased in the other two groups, with the between-group changes for these variables statistically significant for the comparison against each of the other groups ($P<.05$). Similar results were observed in these variables after adjustment for total energy intake. Observed changes were as follows: estimated NEAP using Frasseto equations, in mEq/kcal, $-3.12$ ($-5.42$ to $-0.83$) in the control group, $-1.17$ ($-3.23$ to $0.88$) in the Mediterranean diet with virgin olive oil group, and $0.218$ ($-1.89$ to $2.32$) in the Mediterranean diet with mixed nuts group; estimated NEAP using Remer equations, in mEq/kcal, $-6.22$ ($-10.42$ to $-2.02$) in the control group, $-1.74$ ($-5.52$ to $2.03$) in the Mediterranean diet with virgin olive oil group, and $1.91$ ($-1.95$ to $5.77$) in the Mediterranean diet with mixed nuts group ($P = .02$ vs control group); PRAL, in mEq/kcal, $-6.36$ ($-10.54$ to $-2.17$) in the control group, $-1.73$ ($-5.49$ to $2.03$) in the Mediterranean diet with virgin olive oil group, and $1.85$ ($-1.99$ to $5.71$) in the Mediterranean diet with mixed nuts group ($P = .01$ vs control group).

**Bone Biochemical Changes**

No between-group differences were observed in changes in biochemical bone markers or densitometric bone markers after 1 year of intervention (Table 5). A significantly greater increase in PTH levels was observed in Mediterranean diet with mixed nuts group than in the other two groups. Furthermore, in comparison with the control group, subjects allocated to the Mediterranean diet with nuts showed a tendency to increase urinary levels of free deoxypyridinoline:creatinine ratio after 1 year of nutritional intervention, although between-group differences did not reach statistical significance. No differences in these results were observed even when sex was included in the analysis of variance model (data not shown).
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derly population. The results of the study suggest that a Med-
iterranean dietary pattern enriched with mixed nuts,
although leading to higher dietary acidosis, does not seem
to have any significant effect on bone metabolism.

### DISCUSSION

This is the first interventional study evaluating how a Med-
iterranean diet and a dietary acid load arising from mixed
nuts consumption affect bone health biomarkers in an el-

erly population. The results of the study suggest that a Med-
iterranean dietary pattern enriched with mixed nuts,
although leading to higher dietary acidosis, does not seem
to have any significant effect on bone metabolism.
Observational studies have associated the intake of fruits with higher levels of bone synthesis and lower levels of bone resorption markers. Furthermore, high fruit consumption has been related to higher BMC independent of age, although few interventional studies have evaluated the effect of diet on bone biological markers, and most of them evaluated the effect of only some nutritional components such as isoflavones, carbonated drinks,

### Table 5. Changes in Bone Metabolism Markers at the End of the 12-Month Intervention

<table>
<thead>
<tr>
<th>Bone Metabolism Marker</th>
<th>Control Diet Group (n = 59)</th>
<th>Mediterranean Diet with Virgin Olive Oil Group (n = 73)</th>
<th>Mediterranean Diet with Mixed Nuts Group (n = 70)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass index, kg/m²</td>
<td>0.24 (– 0.07–0.55)</td>
<td>– 0.06 (– 0.26–0.13)</td>
<td>0.10 (– 0.10–0.29)</td>
<td>.18</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>0.33 (– 0.61–1.28)</td>
<td>– 0.41 (– 1.08–0.26)</td>
<td>0.35 (– 0.38–1.08)</td>
<td>.27</td>
</tr>
<tr>
<td>Bone mineral density, g/cm²</td>
<td>0.03 (– 0.05 to – 0.00)</td>
<td>– 0.01 (– 0.04–0.01)</td>
<td>– 0.02 (– 0.03 to – 0.00)</td>
<td>.65</td>
</tr>
<tr>
<td>Broadband ultrasound attenuation, dB/MHz</td>
<td>– 2.01 (– 5.89–1.85)</td>
<td>– 1.41 (– 5.3–2.54)</td>
<td>– 0.57 (– 2.86–1.66)</td>
<td>.84</td>
</tr>
<tr>
<td>Speed of sound, m/s</td>
<td>– 5.40 (– 9.73 to – 1.06)</td>
<td>– 3.41 (– 9.38–2.54)</td>
<td>– 6.38 (– 10.07 to – 2.69)</td>
<td>.66</td>
</tr>
<tr>
<td>Serum calcium, mg/dL</td>
<td>– 0.03 (– 0.14–0.07)</td>
<td>– 0.00 (– 0.14–0.13)</td>
<td>– 0.04 (– 0.16–0.06)</td>
<td>.88</td>
</tr>
<tr>
<td>Parathyroid hormone, pg/mL</td>
<td>– 2.70 (– 6.56–1.16)</td>
<td>– 4.24 (– 7.50 to – 0.99)</td>
<td>2.63 (– 1.09–6.35)</td>
<td>.02</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>2.12 (– 2.43–6.67)</td>
<td>0.51 (– 2.83–3.85)</td>
<td>1.23 (– 1.85–4.32)</td>
<td>.83</td>
</tr>
<tr>
<td>Bone-specific alkaline phosphatase, %</td>
<td>– 1.94 (– 4.46–0.58)</td>
<td>0.48 (– 2.59–1.62)</td>
<td>0.99 (– 1.40–3.39)</td>
<td>.22</td>
</tr>
<tr>
<td>Osteoprotegerin, pmol/L</td>
<td>0.10 (– 0.17–0.37)</td>
<td>– 0.04 (– 0.17–0.16)</td>
<td>0.21 (– 0.06–0.48)</td>
<td>.42</td>
</tr>
<tr>
<td>Deoxypyridinoline:creatinine, nmol/mmol</td>
<td>– 0.36 (– 0.84–0.12)</td>
<td>0.007 (– 0.45–0.46)</td>
<td>0.31 (– 0.13–0.74)</td>
<td>.14</td>
</tr>
<tr>
<td>Urinary calcium:creatinine, mg/mg</td>
<td>– 0.002 (– 0.016–0.012)</td>
<td>– 0.013 (– 0.03–0.001)</td>
<td>– 0.008 (– 0.02–0.007)</td>
<td>.53</td>
</tr>
<tr>
<td>Urine pH</td>
<td>– 0.03 (– 0.24–0.16)</td>
<td>– 0.035 (– 0.23 to – 0.16)</td>
<td>– 0.06 (– 0.20–0.08)</td>
<td>.97</td>
</tr>
</tbody>
</table>

P < .05 versus low-fat diet group; b .001 versus Mediterranean diet with virgin olive oil group.

Figure 1. Flow of subjects through the trial.
protein intake, sodium, and calcium and vitamin D supplements. Recently, the effect of a healthy dietary pattern (Dietary Approaches to Stop Hypertension (DASH) diet approach) was studied in 186 subjects using a 30-day interventional clinical trial on bone metabolism. After intervention, significantly lower osteocalcin (OC) and C-terminal telopeptide of type I collagen (CTX) was reported in the DASH diet group than in the control group. The Mediterranean diet is also considered to be a healthy nutritional pattern mainly because of the high intake of fruits, vegetables, and virgin olive oil; moderate consumption of fish and wine; and low consumption of meat and dairy products, but in contrast to the results obtained with the DASH diet, the results of the current study do not support that the Mediterranean diet benefits densitometric or biochemical bone markers. These discrepancies could be because the differences between the control diet and the Mediterranean diet in the current study were mainly focused on lipid content. Whereas overall fat intake was lower in the American Heart Association diet that was recommended to the control diet group, the Mediterranean diet provides ad libitum sizable amounts of unsaturated fats, such as those contained in extra virgin olive oil and nuts. No other significant changes related to the rest of food or nutrient intake were observed after dietary intervention, and this is probably one of the most important limitations of this study. This small effect of the intervention can be explained, because the participants were older, community-living persons, and they had already been eating a reasonably good Mediterranean diet pattern for a long time before randomization into the trial; thus, the small nutritional changes that were achieved after 1 year of nutritional intervention did not have enough magnitude of effect to be able to improve resorption markers in the Mediterranean diet with virgin olive oil group. During the intervention, only in subjects on the Mediterranean diet receiving a supplement of mixed nuts was the deoxypyridinoline:creatinine ratio nonsignificantly higher and PTH levels significantly higher than in the other two groups. This could be partially explained because nuts are rich in PUFA. There is recent evidence from a case-control study conducted in Spain that high intake of PUFA is associated with greater risk of osteoporotic fractures, whereas a high mono-unsaturated fatty acid:PUFA ratio was protective. This is consistent with findings of a tendency toward a detrimental effect on bone resorption markers associated with Mediterranean diet with mixed nuts, although the results are far from conclusive.

It has been suggested that bone mass acts as a buffer base directed to neutralize the acid load produced by mixed diets, and a diet favoring “alkaline ash,” emphasizing the ingestion of fruits and vegetables and a moderate intake of milk has been proposed to prevent and treat osteoporosis. In 1918, bone depletion in rabbits fed with acid diets was described for the first time. Since then, some studies have related systemic acidosis to osteomalacia and bone mass loss in animals and humans with chronic renal failure. There is a general consensus that diet can modify acid–base status. An established method for estimating acid loads of foods or diets is to calculate dietary PRAL or daily NEAP with or without the contribution of organic acids. Fruits, vegetables, and red wine are all components of the Mediterranean food pattern and are likely to provide an alkaline load, according to the calculated base-forming potential of foods, but among tree nuts, those that provide a significant acid load are peanuts, walnuts, and almonds (8.3, 6.8, and 0.4 mEq/100 g, respectively), whereas other tree nuts such as hazelnuts give a basic load (−2.8 mEq/100 g) probably because of their higher calcium and magnesium content and slightly lower levels of protein and phosphorous. The data from the current study show that foods are important determinants of dietary acid load. Thus, PRAL is predominantly basic in the Mediterranean diet with virgin olive oil and in the control group, in which intake of fruits, vegetables, and red wine increased. Alternatively, subjects in the Mediterranean diet with mixed nuts group had a significant increase in PRAL and NEAP, indicating a high dietary acid load probably associated with the greater consumption of nuts and almonds. However, no significant correlation was observed between changes in bone markers and changes in PRAL or NEAP (data not shown), and no significant differences in bone markers were observed in subjects on the Mediterranean diet with nuts group and the other two groups, except for PTH levels and a nonsignificantly greater deoxypyridinoline:creatinine ratio.

In contrast to the results of the current study, a recent study described a weak relationship between changes in PTH levels and changes in renal acid net excretion in a small group of subjects. Furthermore, a cross-sectional study of premenopausal women found that subjects in the highest quartile of energy-adjusted NEAP estimated from FFQs showed higher pyridinoline and deoxypyridinoline excretion than those in lower quartiles. High net renal excretion has also been associated with lower femoral neck BMD and lower lumbar spine BMD, and higher acidic dietary load (high PRAL) has been inversely associated with lower calcaneal BUA in women, but not in men, even after adjustment for energy intake and other confounding variables. The current study failed to observe any significant differences in bone densitometric parameters between the groups, although a slight tendency toward lower BUA was observed in the Mediterranean diet with nuts group, that is, those with higher PRAL, than in the other groups. The design of the study and the method used to evaluate osteoporosis could explain these results. Because changes in BMD and other bone densitometric markers take place slowly, (an estimated 1% of bone mass is lost per year in postmenopausal women, and even less is lost in men) the 1-year treatment was probably insufficient to detect changes in bone mass and microstructural markers. Furthermore, the predictive value of quantitative ultrasound parameters for BMD is limited, particularly with only 1 year of follow up. Dual-energy X-ray absorptiometry is more reliable than quantitative ultrasound technology in predicting BMD, although limited precision would not necessarily prevent detection of large changes that were much greater than the observed variance in the test. Finally, the subject selection criteria required for the PREDIMED Study when cardiovascular mortality is the end point, focus on subjects with a high risk of CVD with type 2 diabetes mellitus or at least three other risk factors for CVD such as hyperlipidemia, hypertension, and medical therapy for these conditions. These subject selection criteria could limit the generalizability of the findings.
In conclusion, the results of this study do not support that the Mediterranean diet or added nuts have any beneficial effect on bone health biomarkers over the low-fat diet recommended by the American Heart Association. Furthermore, the results do not support the hypothesis that a dietary acid load could activate the compensatory acid-base mechanisms and thus exert a deleterious effect on bone health. Whether the magnitude of dietary acid load results in osteopenia or osteoporosis remains to be elucidated. Therefore, future studies will be needed to demonstrate equivalence between these interventions, preferably using a specific design for noninferiority (equivalence) trials. Absence of evidence does not mean evidence of no effect. To demonstrate evidence of no effect, an equivalence trial would be needed, which requires different techniques during design and analysis, including the establishment of the margin of equivalence and the choice of the type of test.

ACKNOWLEDGMENTS
Conflict of Interest: Dr. Jordi Salas-Salvadó is an unpaid member of the Scientific Advisory Board of the Internat Nut Council. The rest of authors do not have any conflict of interest affecting the conduct or reporting of the work submitted.

Author Contributions: Mònica Bulló and Jordi Salas: study design, study performance, data analysis, and writing of the manuscript. Josep Basora and Rosa Sola: coordinators of subject recruitment at the outpatient clinics. Pilar Amigó-Correig, Fabiola Márquez-Sandoval, and Nancy Babio: dietitians controlling the intervention diets. Miguel A. Martínez-González and Ramon Estruch: PREDIMED Study design.

Sponsor’s Role: Spanish Ministry of Health (Instituto de Salud Carlos III, Thematic Network G03/140 and RTIC RD06/0045, Fondo de Investigaciones Sanitarias, PI04/0233, PI04/1828, PI04/2239, and PI05/2368; PI05/1839), CYCYT AG2005-0365, Public Health Division of the Department of Health of the Autonomous Government of Catalonia, and Fundación Patrimonio Comunal Olivarero and Hojiblanca SA (Málaga, Spain), California Walnut Commission (Sacramento, CA), Borges SA (Reus, Spain), and Morella Nuts SA (Reus, Spain) donated the olive oil, walnuts, almonds, and hazelnuts, respectively, used in the study. None of the funding sources played a role in the design, collection, analysis, or interpretation of the data or in the decision to submit the manuscript for publication.

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