Dihydrophylloquinone intake is associated with low bone mineral density in men and women\textsuperscript{1–4}

Lisa M Troy, Paul F Jacques, Marian T Hannan, Douglas P Kiel, Alice H Lichtenstein, Eileen T Kennedy, and Sarah L Booth

ABSTRACT

Background: Poor diet may affect bone status by displacing nutrients involved in bone health. Dihydrophylloquinone, a form of vitamin K present in foods made with partially hydrogenated fat, is a potential marker of a low-quality dietary pattern.

Objective: Our objective was to examine the cross-sectional associations between dihydrophylloquinone intake and bone mineral density (BMD) of the hip and spine in men and women.

Design: Dihydrophylloquinone intake was estimated with a food-frequency questionnaire, and BMD (in g/cm\textsuperscript{2}) was measured by dual-energy X-ray absorptiometry in 2544 men and women (mean age: 58.5 y) who had participated in the Framingham Offspring Study. General linear models were used to examine the associations between dihydrophylloquinone intake (in tertiles: <15.5, 15.5–29.5, and >29.5 µg/d) and hip and spine BMD after adjustment for age, body mass index, energy intake, calcium intake, vitamin D intake, smoking status, physical activity score, and, for women, menopause status and estrogen use.

Results: Higher dihydrophylloquinone intakes were associated with lower mean BMD at the femoral neck [lowest-to-highest tertiles (95% CI): 0.934 (0.925, 0.942), 0.927 (0.919, 0.935), and 0.917 (0.908, 0.926), \(P\) for trend = 0.02], the trochanter [lowest-to-highest tertiles (95% CI): 0.811 (0.802, 0.820), 0.805 (0.797, 0.813), and 0.795 (0.786, 0.804), \(P\) for trend = 0.02], and the spine [lowest-to-highest tertiles (95% CI): 1.250 (1.236, 1.264), 1.243 (1.242, 1.229), and 1.227 (1.213, 1.242), \(P\) for trend = 0.03] in men and women after adjustment for the covariates. Further adjustment for markers of healthy and low-quality dietary patterns did not affect the observed associations.


KEY WORDS Dihydrophylloquinone, vitamin K, partially hydrogenated fat, trans fatty acids, diet patterns, bone mineral density, osteoporosis

INTRODUCTION

Evidence indicates that several foods, such as fruit and vegetables, and nutrients beyond calcium and vitamin D, such as phylloquinone (vitamin K\(_1\)), positively influence bone status (1–4). Conversely, the components of a low-quality dietary pattern, such as sweet baked products and candy, have been shown to be associated with lower bone mineral density (BMD; 5), possibly by displacing foods that contain important nutrients involved in bone health.

Dihydrophylloquinone is formed when phylloquinone-rich plant oils are synthetically hydrogenated. Because much of the vegetable oil consumed in the United States is in the partially hydrogenated form, dihydrophylloquinone is a common source of vitamin K and contributes as much as 30% of total vitamin K intake (6). However, unlike phylloquinone, which is found in foods associated with a healthy dietary pattern, the major dietary sources of dihydrophylloquinone are commercially baked snack and fried foods (6, 7). Consequently, dihydrophylloquinone is a marker of snack food and fast food intakes, which are elements of a low-quality dietary pattern.

We hypothesized that higher intakes of dihydrophylloquinone, as a marker of a low-quality dietary pattern, are associated with lower BMD. To test this hypothesis, we examined cross-sectional associations between dihydrophylloquinone intake and BMD of the hip and spine in men and women.

SUBJECTS AND METHODS

Subjects

The Framingham Offspring Study is a longitudinal community-based study that began in 1971, with the primary focus of the study being to identify factors that contribute to the development of cardiovascular disease and associated risk factors. The study is conducted by the National Heart, Lung, and Blood Institute’s Framingham Heart Study (NO1-HC-25195); and the US Department of Agriculture under agreement 58-1950-001.

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2 Any opinions, findings, conclusions or recommendations expressed in this publication are those of the authors and do not necessarily reflect the views of the US Department of Agriculture.

3 Supported by the National Institute of Aging (AG14759 SLB); the National Institute of Arthritis and Musculoskeletal and Skin Diseases (AR/AG 41398 DPK); the National Heart, Lung, and Blood Institute’s Framingham Heart Study (NO1-HC-25195); and the US Department of Agriculture under agreement 58-1950-001.

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Received January 3, 2007.

Accepted for publication April 9, 2007.
goal of evaluating heart disease risk factors in 5124 adult off-
spring (and the offspring’s spouses) of the original Framingham
Heart Study cohort participants. Follow-up study visits occur
every 3–4 y and include physical examinations, anthropometric
measurements, biochemical assessments, questionnaires, and
the evaluation of cardiovascular disease and other risk factors.
For dietary information, participants were mailed a food-
frequency questionnaire (FFQ) and were asked to bring the com-
pleted FFQ with them to the sixth (1995–1998) and seventh
(1998–2001) follow-up examinations. The Framingham Osteo-
porosis Study, an ancillary study to the Framingham Offspring
Study, conducted baseline BMD assessments during 1996–2001
to collect osteoporosis-related information in the Framingham
Offspring Study cohort.

BMD measurements, valid FFQ data, and covariates were
available for 2636 Framingham Osteoporosis Study participants.
For purposes of the present analysis, we excluded 92 participants
who reported using warfarin or other oral anticoagulants, which
act as vitamin K antagonists, osteoporosis medications, or both
at the same time as the BMD measurements were obtained. A total
of 2544 subjects (1130 men and 1414 women) remained and
were included in the analyses. The present study was approved by
the institutional review boards of Hebrew Senior Life, Tufts–
New England Medical Center, and Boston University School of
Medicine.

Dietary assessment

Usual dietary intake over the previous year was reported with
the use of the Willett FFQ. The FFQ consisted of a list of 126 food
items with specified standard serving sizes and a selection of 9
frequency of consumption categories, which ranged from never
or <1 serving/mo to 6 servings/d, as described elsewhere (8, 9).
We excluded from the analyses those subjects who reported an
energy intake <600 kcal/d (2.51 MJ/d) or >4000 kcal/d (16.74
MJ/d) for women or 4200 kcal/d (17.57 MJ/d) for men. Subjects
who left ≥12 items blank on the FFQ were also excluded.

Daily dietary dihydrophyloquinone intake was calculated by
multiplying the dihydrophyloquinone content per serving of
each food (6, 10, 11) by the reported frequency of consumption
from the FFQ and summing over all foods. The dihydrophylo-
quinone content of foods was based on laboratory analyses of
geographically represented foods collected as part of the US
Department of Agriculture National Food and Nutrient Analyses
Plan, as described elsewhere (10). We previously reported that a
higher dihydrophyloquinone intake was associated with higher
plasma dihydrophyloquinone concentrations and with higher
trans fatty acid intakes in the Framingham Offspring Study (12).

Various measures of diet quality were calculated. Baked,
fried, and snack foods, which reflect a low-quality dietary pat-
tern, were split into 2 categories: commercially baked, fried, and
snack foods that contained dihydrophyloquinone (brownies,
cakes, chips, cookies, crackers, doughnuts, French fries, muffins
or biscuits, pies, popcorn, and sweet rolls) and snack foods that
were unlikely to contain dihydrophyloquinone (candy and nuts).
The number of servings of baked, fried, and snack foods per day
was calculated by summing the number of servings per day of
each of these food items from the FFQ. Fruit and vegetable
intake, and magnesium and potassium intakes, which are found
primarily in whole and unrefined foods, reflect a healthy dietary
pattern. The number of servings of fruit and vegetables per day
was calculated by summing the number of servings per day of
canned, fresh, and frozen fruit and vegetables from the FFQ.
Magnesium and potassium intakes were calculated by summing
the contribution of magnesium or of potassium from each food
item from the FFQ. In addition, we calculated a measure of
overall diet quality, the 2005 Dietary Guidelines Adherence In-
dex. The 2005 Dietary Guidelines Adherence Index, as described
elsewhere (13), was developed to assess the adherence to key
dietary intake recommendations of the US government’s 2005
Dietary Guidelines for Americans, which describes a dietary
pattern intended to promote health and to prevent chronic disease
(14). The possible range of the 2005 Dietary Guidelines Adher-
ence Index scores is a minimum of 0 to a maximum of 20.

Bone mineral density

BMD of the proximal right femur, which includes the femoral
neck and the trochanter, and the lumbar spine (L2–L4) was mea-
sured by dual-energy X-ray absorptiometry and with standard
positioning as recommended by the manufacturer (DPX-L;
Lunar, Madison, WI). The right hip region was scanned unless
there was a history of previous fracture or hip joint replacement,
in which case the left hip was scanned. The CVs for the DPX-L
measurements were 1.7% for the femoral neck, 2.5% for the
trochanter, and 0.9% for the lumbar spine (15). Monthly mea-
surements of a bone phantom throughout the period of BMD data
collection showed no machine drift.

Covariate information

Potential factors that affect BMD status were identified, and
information was collected either at the time of the BMD scans or
at a date closest in time to both the Framingham and BMD
examinations. The factors included age; sex; weight; height; total
daily intakes of energy, calcium, and vitamin D (diet plus sup-
plements); alcohol intake (number of drinks per day); current
cigarette smoking status (smoked regularly in the past year: yes
or no); and physical activity as estimated by the Physical Activity
Scale for the Elderly (PASE). PASE is a 7-d record of household
activities (eg, housework) and the number of hours spent on usual
daily activities (eg, standing) and on light, moderate, and strenuous
sports and recreation. PASE is a commonly used brief survey to
assess physical activity in the elderly (16). For women, current
hormone replacement therapy (HRT; reported use of oral conjug-
gated estrogen, patch, or cream: yes or no) and menopause status
(defined as no menstrual bleeding for 1 y, reported current HRT, or
age ≥55 y) were also included as covariates. Body mass index
(BMI; in kg/m²) was calculated from weight and height recorded at
the closest follow-up examination to the BMD measurements.

Statistical methods

All the statistical analyses were performed with the Statistical
Analysis System (version 9.1; SAS Institute Inc, Cary, NC). The
analyses were conducted for men and women together because
there was no significant interaction between sex and dihydro-
phyloquinone intake. Statistical significance refers to \( P < 0.05 \)
unless otherwise noted.

All analyses used tertiles of dihydrophyloquinone intake
(<15.5, 15.5–29.5, >29.5 \( \mu \)g/d), which were based on the in-
takes reported by the entire cohort. Least-squares means from the
general linear models procedure (PROC GLM, version 9.1; SAS
Institute Inc) were used to examine the descriptive comparisons
by tertile of dihydrophyloquinone intake. The resulting
least-squares means for each of the subject characteristics and dietary measures were compared across all pairwise combinations with the use of the Tukey-Kramer option to adjust for multiple comparisons in the GLM procedure.

Least–squares mean BMD at the femoral neck, trochanter, and lumbar spine (L2–L4) were calculated overall and by tertile of dihydrophylloquinone intake, with adjustment for the covariates age; BMI; smoking status; physical activity score; intakes of total energy, calcium and vitamin D (diet plus supplements); alcohol intake; and, for women, menopause status and current HRT. In addition, a test for linear trend by tertile of dihydrophylloquinone intake was performed by assigning the median value of dihydrophylloquinone to each tertile and by treating these median intakes listed above. To assess the influence of calcium and phylloquinone intake for each tertile and by treating these median values as continuous variables, after adjustment for the covariates (Table 2). Similar trends were observed after additional analyses that controlled for markers of diet quality, which include the 2005 Dietary Guidelines Adherence Index and the overall healthy dietary pattern (as estimated by the 2005 Dietary Guidelines Adherence Index; 13) and for an independent marker of a low-quality dietary pattern, we conducted additional analyses that controlled for markers of a healthy dietary pattern (number of fruit and vegetable servings, potassium intake, and magnesium intake separately) and of an overall healthy dietary pattern (as estimated by the 2005 Dietary Guidelines Adherence Index: 13) and for an independent marker of a low-quality dietary pattern (snack foods unlikely to contain dihydrophylloquinone) in GLM models.

RESULTS

Subject characteristics are summarized in Table 1 as overall means ± SEMs (unless otherwise noted) by tertile of dihydrophylloquinone intake. Men and women had a mean age of 58.5 ± 0.5 and a mean BMI in the overweight and obese classification (BMI > 25). Forty-one percent of the entire population was overweight (BMI: 25-30), and 28% was obese (BMI > 30). Eighty-one percent of the women were postmenopausal (n = 1139), 37% of whom were receiving HRT (n = 422) at the time these data were collected.

The major finding of the present study was that higher dihydrophylloquinone intake was significantly associated with lower femoral neck, trochanter, and lumbar spine (L2–L4) BMD in

DISCUSSION

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### TABLE 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n = 2544)</th>
<th>&lt;15.5 (n = 853)</th>
<th>15.5–29.5 (n = 856)</th>
<th>&gt;29.5 (n = 835)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>58.5 ± 0.2</td>
<td>58.5 ± 0.3</td>
<td>58.5 ± 0.3</td>
<td>58.5 ± 0.3</td>
</tr>
<tr>
<td>Male (%)</td>
<td>44.4</td>
<td>35.4</td>
<td>45.3</td>
<td>52.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.9 ± 0.1</td>
<td>27.3 ± 0.2</td>
<td>28.1 ± 0.2</td>
<td>28.3 ± 0.2</td>
</tr>
<tr>
<td>PASE</td>
<td>145 ± 2</td>
<td>146 ± 3</td>
<td>146 ± 3</td>
<td>144 ± 3</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>13.7</td>
<td>12.6</td>
<td>14.3</td>
<td>14.1</td>
</tr>
<tr>
<td>Energy (kcal/d)</td>
<td>1842 ± 12</td>
<td>1493 ± 18</td>
<td>1796 ± 18</td>
<td>2245 ± 18</td>
</tr>
<tr>
<td>Dihydrophylloquinone (µg/d)</td>
<td>27.1 ± 0.4</td>
<td>9.6 ± 0.4</td>
<td>22.0 ± 0.4</td>
<td>50.3 ± 0.4</td>
</tr>
<tr>
<td>Calcium (mg/d)</td>
<td>922 ± 10</td>
<td>888 ± 17</td>
<td>891 ± 17</td>
<td>989 ± 17</td>
</tr>
<tr>
<td>Vitamin D (IU/d)</td>
<td>395 ± 6</td>
<td>407 ± 10</td>
<td>379 ± 10</td>
<td>398 ± 10</td>
</tr>
<tr>
<td>Phylloquinone (µg/d)</td>
<td>164 ± 2</td>
<td>162 ± 4</td>
<td>163 ± 4</td>
<td>167 ± 4</td>
</tr>
<tr>
<td>Fruit and vegetables (servings/d)</td>
<td>4.5 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>4.5 ± 0.1</td>
<td>4.6 ± 0.1</td>
</tr>
</tbody>
</table>
| Baked, fried, and snack foods (servings/d) | 2.2 ± 0.1 | 1.0 ± 0.1* | 1.9 ± 0.1* | 3.8 ± 0.1*

' n = 1130 men and 1414 women. PASE, Physical Activity Score for the Elderly (observed range: 0–417). Least-squares means (unadjusted) in each tertile calculated by general linear models procedure (PROC GLM, version 9.1; SAS Institute Inc, Cary, NC). Values in the same row with different superscript letters are significantly different. P < 0.05 (Tukey-Kramer pairwise comparison test).

2 ± SEM (all such values).

3 Diet plus supplements.
DIHYDROPHYLLOQUINONE INTAKE AND BMD

Least-squares mean BMD measurements for general linear models (GLM), by tertile of dihydrophylloquinone intake for men and women

<table>
<thead>
<tr>
<th>Bone site</th>
<th>Tertile of dihydrophylloquinone intake (µg/d)</th>
<th>g/cm²</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;15.5 (n = 853)</td>
<td>15.5–29.5 (n = 856)</td>
<td>&gt;29.5 (n = 835)</td>
</tr>
<tr>
<td>Standard model†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.934 ± 0.005† 0.004</td>
<td>0.927 ± 0.004</td>
<td>0.917 ± 0.005</td>
</tr>
<tr>
<td>Trochanter</td>
<td>0.811 ± 0.004</td>
<td>0.805 ± 0.004</td>
<td>0.795 ± 0.004</td>
</tr>
<tr>
<td>Spine (L2-L4)</td>
<td>1.250 ± 0.007</td>
<td>1.243 ± 0.006</td>
<td>1.227 ± 0.007</td>
</tr>
<tr>
<td>DGA1 model‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.936 ± 0.005</td>
<td>0.927 ± 0.004</td>
<td>0.919 ± 0.005</td>
</tr>
<tr>
<td>Trochanter</td>
<td>0.814 ± 0.005</td>
<td>0.805 ± 0.004</td>
<td>0.795 ± 0.005</td>
</tr>
<tr>
<td>Spine (L2-L4)</td>
<td>1.251 ± 0.008</td>
<td>1.243 ± 0.007</td>
<td>1.230 ± 0.008</td>
</tr>
<tr>
<td>Other snack foods model§</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Femoral neck</td>
<td>0.930 ± 0.004</td>
<td>0.924 ± 0.004</td>
<td>0.914 ± 0.005</td>
</tr>
<tr>
<td>Trochanter</td>
<td>0.805 ± 0.004</td>
<td>0.798 ± 0.004</td>
<td>0.789 ± 0.04</td>
</tr>
<tr>
<td>Spine (L2-L4)</td>
<td>1.246 ± 0.007</td>
<td>1.240 ± 0.006</td>
<td>1.225 ± 0.007</td>
</tr>
</tbody>
</table>

† Least-squares means calculated by general linear models procedure (PROC GLM, version 9.1; SAS Institute Inc, Cary, NC) and adjusted for the covariates age, BMI, sex; total intakes of energy, calcium, and vitamin D (diet plus supplements); alcohol intake; current smoking status; Physical Activity Score for the Elderly; and, for women, menopause status (yes or no) and current hormone replacement therapy (yes or no).

‡ Standard model + the 2005 Dietary Guidelines Adherence Index (DGA1; 13)—an overall healthy dietary pattern, adjusted for covariates.

§ Standard model + snack foods unlikely to contain dihydrophylloquinone—a marker of a low-quality dietary pattern, adjusted for covariates.

men and women. This observation is consistent with a previous study that examined food groups, in which the participants who reported consuming sweet baked products, a primary source of dihydrophylloquinone, had lower BMD measures than did the individuals who had a healthy dietary pattern (5). However, in the current study, the number of fruit and vegetable servings was not lower with higher dihydrophylloquinone intakes, which suggests that baked, fried, and snack food intakes did not displace fruit and vegetable intakes.

Our findings do not support the hypothesis that the association between dihydrophylloquinone intake and BMD is due to dietary pattern. The significant associations between higher dihydrophylloquinone intake and lower BMD remained after additional adjustment for multiple markers of healthy and low-quality dietary patterns. Instead, the observed inverse associations between dihydrophylloquinone intake and BMD is due to dietary patterns. The significant associations between higher dihydrophylloquinone intake and BMD appears to be independent of diet quality. The data from the present study suggest that dihydrophylloquinone intake may have a detrimental effect on bone status or may act as a marker of other dietary or lifestyle factors not captured in our assessment of dietary patterns.

We are grateful to the Framingham Study participants and staff, especially the densitometer technician Mary Hogan and the Framingham Study laboratory.
chief Patrice Sutherland and her staff. We are also grateful to Gail Rogers for programming assistance and James Peterson for technical assistance.

The authors’ responsibilities were as follows—LMT: designed and performed the statistical analyses and drafted the manuscript; PFJ, MTH, and DPK: contributed to the design of the analyses, the interpretation of the data, and the writing of the manuscript; AHL and ETK: contributed to the interpretation of the data and the writing of the manuscript; SLB: designed the analyses, interpreted the data, and contributed to the writing of the manuscript; DPK: directs the Framingham Osteoporosis Study; DPK, MTH, and SLB: collected and managed the data used in these analyses; and all authors: reviewed the final manuscript. None of the authors reported any conflicts of interest.

REFERENCES