Ratio of n−6 to n−3 fatty acids and bone mineral density in older adults: the Rancho Bernardo Study1–3

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ABSTRACT
Background: Several lines of evidence suggest that n−3 fatty acids reduce the risk of some chronic diseases, including heart disease, diabetes, and cancer. Other research, mainly in animals, also suggests a role in bone health.

Objective: We aimed to investigate the association between the ratio of dietary n−6 to n−3 fatty acids and bone mineral density (BMD) in 1532 community-dwelling men and women aged 45–90 y.

Design: Between 1988 and 1992, dietary data were obtained through self-administered food-frequency questionnaires, and BMD was measured at the hip and spine with the use of dual-energy X-ray absorptiometry. A medical history was obtained and current medication use was validated. Age- and multiple-adjusted linear regression analyses were performed.

Results: There was a significant inverse association between the ratio of dietary linoleic acid to α-linolenic acid and BMD at the hip in 642 men, 564 women not using hormone therapy, and 326 women using hormone therapy; these results were independent of age, body mass index, and lifestyle factors. An increasing ratio of total dietary n−6 to n−3 fatty acids was also significantly and independently associated with lower BMD at the hip in all women and at the spine in women not using hormone therapy.

Conclusions: A higher ratio of n−6 to n−3 fatty acids is associated with detrimental effects on bone health, and a lower ratio is associated with healthy bone properties (5). Results from a small number of experimental studies of the effects of PUFA supplementation on bone in humans are inconclusive (8–10). The results of a recent investigation by MacDonald et al (11) suggest that PUFAs are harmful to bone in women. No large epidemiologic studies have reported the association between ratios of PUFAs and bone mineral density (BMD) in both sexes. We report here the association between the ratio of dietary n−6 to n−3 fatty acids and BMD in community-dwelling older men and women.

SUBJECTS AND METHODS
Study population
Subjects were participants in the Rancho Bernardo Study, a population-based cohort of older, middle to upper-middle class, white residents of a southern California community. Between 1988 and 1992, all surviving community-dwelling members of this cohort were invited to participate in a study of osteoporosis.

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At this visit, BMD was measured and a food-frequency questionnaire was administered. All participants gave written informed consent. The Institutional Review Board of the University of California, San Diego, approved the protocol.

Assessment of diet

Information on usual dietary intake was obtained from 643 men and 903 postmenopausal women by using the self-administered semiquantitative Harvard-Willett Diet Assessment Questionnaire (12). Self-reported foods were converted into nutrients by using the food-composition database of the US Department of Agriculture. This food-frequency questionnaire was designed to quantitatively individual fatty acids, essential parent compounds, linoleic acid (LA; 18:2n−6) and α-linolenic acid (ALA; 18:3n−3), and the corresponding long-chain fatty acids, arachidonic acid (20:4n−6), eicosapentaenoic acid (20:5n−3), and docosahexaenoic acid (22:6n−3). PUFA ratios were entered into the model separately: LA:ALA and total n−6 (LA + arachidonic acid):total n−3 (ALA + eicosapentaenoic acid + docosahexaenoic acid). Information on smoking habits, alcohol intake, exercise frequency, reproductive history, and use of vitamins, thiazides, thyroid hormones, steroids, and estrogen was also obtained through standard questionnaires. All pills and prescriptions were brought to the study center for confirmation of current supplement and medication use by a specially trained nurse. Height and weight were measured while the participants wore light clothing and no shoes.

Measurement of bone mineral density

Baseline BMD was measured at the total hip and lumbar spine in g/cm² by dual-energy X-ray absorptiometry (QDR model 1000; Hologic Inc, Waltham, MA). Total hip BMD was obtained by summing the bone mineral content at the femoral neck, intertrochanter, and greater trochanter and dividing by the composite area of the 3 sites. Spine BMD was defined as the average BMD of lumbar vertebrae L1–L4. Instruments were calibrated daily and had measurement precisions of ≤1% for the spine and ≤1.5% for the hip.

Data analysis

Complete dietary variables and BMD measurements from the same visit between 1988 and 1992 were available for 642 men and 890 women. Statistical analyses were performed by using SPSS (SPSS for WINDOWS 11.5; SPSS Inc, Chicago, IL). Data from men and women were analyzed separately, and women were further stratified according to hormone therapy (HT) status, after preliminary analyses showed a significant interaction between PUFAs and HT on BMD. Total calcium intake was log transformed to meet the assumptions of statistical tests. Comparisons between men and women and between women receiving or not receiving HT were Bonferroni-corrected for multiple comparisons.

The association between total hip or lumbar spine BMD and PUFA ratios was assessed by multiple regression analysis. Regression models were adjusted for age and were fully adjusted for standard osteoporosis covariates, including age, body mass index, calcium intake (diet plus supplements), current exercise (≥3 times per week), smoking status (never, past, or current), alcohol intake, use of thiazides, and use of thyroid hormones. Body mass index was used as a covariate instead of energy intake to adjust for the greater caloric intakes of heavier individuals, because actual energy intake from the FFQ is difficult to capture (12). Increasing age, smoking, alcohol intake, and calcium deficiency have previously been found to affect the metabolism of fatty acids and bone (13). The linearity of the relation between PUFAs and bone was tested by including a quadratic term in the adjusted regression models. There was no evidence for nonlinearity; therefore, linear models were used. Total vitamin C intake was negatively associated with a lower PUFA ratio. Regression analyses that included antioxidant intake (total vitamin E, vitamin C, and retinol) were performed separately, with no significant effect on the observed associations. Regression models were also performed excluding users of fish-oil supplements (n = 24), with no change in the results. Supplement users were included in all models. All statistical tests were significant at the P < 0.05 level.

RESULTS

The baseline characteristics of the study population are shown in Table 1. The mean age of the men was 72.9 y and that of the women was 72.5 y. The groups were compared with the use of independent t tests. The men were heavier than the women and had higher BMD. Women using HT were significantly younger, were taller, and had higher BMD at the hip and lumbar spine than did nonusers. The mean dietary intakes of PUFA variables in men and women are shown in Table 2. Men had a significantly higher ratio of n−6 to n−3 fatty acids than did women. Dietary PUFA intake did not vary significantly by hormone use (Table 2).

Both the age-adjusted and the fully adjusted linear regression analyses for PUFA ratios and BMD at the hip and lumbar spine are shown in Table 3. In the age-adjusted models, the ratios were significantly and negatively associated with hip BMD in all groups and with lumbar spine BMD in women not using HT only (P < 0.05). After adjustment for all covariates, an increasing ratio of LA:ALA remained significantly associated with lower BMD at the hip in men (P < 0.05), women using HT (P < 0.05), and women not using HT (P = 0.05); in this last group, this ratio was also inversely associated with BMD at the spine (P < 0.05). In women with and without HT, the total ratio of n−6 to n−3 fatty acids was inversely associated with hip BMD and was additionally associated with BMD at the spine in women not using HT (P < 0.05).

DISCUSSION

In this cohort, an increasing ratio of dietary n−6 to n−3 fatty acids was significantly associated with lower BMD. The most consistent association was seen with the ratio of LA, the predominant n−6 fatty acid, to ALA, the predominant n−3 fatty acid, where an increasing ratio of n−6 to n−3 fatty acids was associated with lower BMD at the hip in men and in women regardless of HT status and at the lumbar spine in women not using HT. In addition, the ratio of total n−6 to n−3 fatty acids was inversely associated with BMD at the hip in both groups of women and at the lumbar spine in women not using HT. These associations were independent of other bone-related variables, such as age, lifestyle, and medication use.

The average intake of total n−3 fatty acids by the men and women in the current study was 1.3 g/d. This is slightly lower than the average US intake of ∼1.6 g/d. The average ratio of total n−6 to n−3 fatty acids in the present study was 8.4 in men and
7.9 in women. This is also lower than the average ratio of all n-6 to n-3 PUFAs of ~9:8:1 (4). Although the “optimal” ratio of n-6 to n-3 fatty acids is unknown, Paleolithic nutrition studies show that hunter-gatherer populations consumed nearly equal amounts of n-6 and n-3 fatty acids (14). Modern agricultural practices and changes in food processing have been blamed for the increased consumption of n-6 fatty acids through increased intake of corn, sunflower, and sesame oils. At the same time, the intake of n-3 fatty acids decreased as the result of reduced consumption of cold-water fish, changes in animal production practices, and loss of cereal germ in processed grains. Overall, these changes led to an increase in the ratio of n-6 to n-3 fatty acids (4, 14).

Only a few studies have reported the association between PUFAs and bone density in humans, with inconsistent findings. In a cross-sectional study, Terano (15) compared the BMD of 132 men and women aged 38–80 y living in a fishing village in Japan with that of 332 age-matched urban control subjects and found that the women living in the fishing village, who consumed larger amounts of n-3 fatty acids, had greater radial BMD than did the controls. In a recent investigation, MacDonald et al (11) found that PUFA intake was associated with bone loss at the femoral neck in 891 women who consumed low amounts of calcium. In an 18-mo clinical trial in 65 postmenopausal women, Kruger et al (9) found that supplementation with a 10:1 ratio of n-6 to n-3 fatty acids preserved BMD at the spine and increased BMD at the femoral neck, whereas the placebo group experienced bone loss. In 21 of these women, continued supplementation for another 18 mo resulted in significant BMD increases at the lumbar spine and further increases at the femoral neck. Van Papendorp et al (8) found that supplementation with both fish oil and a mixture of fish oil plus
evening primrose oil in a ratio of 1:10:1 for 16 wk increased bone formation and calcium absorption in 40 osteoporotic women. In a clinical trial in 33 postmenopausal women with a mean age of 58.2 y, Terano (15) found that n-3 fatty acid supplementation increased bone density and bone formation compared with placebo.

In many animal studies, n-3 fatty acids or a low ratio of n-6 to n-3 fatty acids has shown a positive influence on bone (7). Studies have shown that differing ratios of n-6 to n-3 fatty acids alter the synthesis of prostaglandin and insulin-like growth factor I (2), that high levels of n-6 fatty acids increase prostaglandin E2 (PGE2) production (16, 17), and that supplementation with n-3 fatty acids or lower ratios of n-6 to n-3 fatty acids increase calcium transport (18) and calcium absorption (13). PUFA-deficient rats were shown to develop severe osteoporosis (13), and a recent study found that n-3 fatty acid repletion re-establishes the ratio of n-6 to n-3 fatty acids in bone compartments and reverses the compromised bone structural properties in n-3-deficient rats (19). In contrast, 3 animal studies showed adverse effects or no effect of n-3 fatty acids or a low ratio of n-6 to n-3 fatty acids on bone in growing rats (20), pigs (21), and rabbits (22).

There are a plethora of biologically plausible pathways whereby PUFAs may regulate the factors involved in bone metabolism, such as prostaglandins, cytokines, insulin-like growth factor I, and calcium. Reviewers have suggested that one or a combination of these factors may have an effect on bone (5, 6, 13, 23). For example, PGE2, the major prostaglandin involved in bone metabolism, is synthesized from n-6 fatty acids, whereas n-3 fatty acids inhibit its production (1, 13). Normal or moderate concentrations of PGE2 support bone formation, whereas greater quantities promote bone resorption (5). Fatty acids are also involved in calcium metabolism. Higher n-3 fatty acid intake enhances calcium absorption, decreases calcium loss, and increases bone calcium (13, 20, 23). In addition, the inhibition of cytokine production has been implicated as a potential mechanism of the favorable effects of fatty acids on bone, with higher intakes of n-3 fatty acids inhibiting the synthesis of proinflammatory cytokines, such as interleukin 6, interleukin 1, and tumor necrosis factor α (24, 25). Kettler (6) suggested that bone loss is mediated by cytokines, and n-3 fatty acid supplementation in animals and humans reduces cytokine synthesis and increases calcium absorption.

In the present study, there was a significant interaction between hormone use and the ratio of dietary n-6 to n-3 fatty acids on BMD at the hip and spine. Fatty acids could potentiate the effect of HT on bone through increasing calcium absorption (26). A study in ovariectomized rats showed that estrogen plus a combination of n-6 and n-3 fatty acids increases bone formation and decreases bone resorption, whereas estrogen alone only increases bone formation (27).

To our knowledge, this is the first large epidemiologic investigation of the association between PUFAs and BMD in older, community-dwelling white men and women who had a wide range of dietary n-6 and n-3 fatty acid intake. The latest longitudinal study by Macdonald et al (11) investigated the association between total PUFAs and bone in women only and did not differentiate between various types of PUFAs (eg, n-3 versus n-6). Previous experimental studies had limited ability to assess

### TABLE 3
Linear regression analysis of the ratio of n-6 to n-3 polyunsaturated fatty acids and bone mineral density in men and women

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1 Participant numbers may vary slightly at different bone sites because of problems of positioning, hip replacement, and participant fatigue. +HT, women currently using hormone therapy; +HT, women currently using hormone therapy; LA, linoleic acid; ALA, α-linolenic acid; total n-6, LA + arachidonic acid; total n-3, ALA + eicosapentaenoic acid + docosahexaenoic acid. β is the unstandardized regression coefficient.

2 Adjusted for age, BMI, total calcium intake, exercise, smoking, alcohol intake, use of thiazides, and use of thyroid hormones.
a range of fatty acid intakes because of study design and small sample sizes.

Although the food-frequency questionnaire is useful for ranking individuals on the basis of their annual dietary intakes, it is semiquantitative and subject to recall bias, particularly for seasonal foods (12, 28). Nevertheless, the reliability of the food-frequency questionnaire for the dietary assessment of PUFAs was validated previously by studies showing a good agreement between fatty acid intake estimated from a food-frequency questionnaire and biological markers of intake (29, 30). In another study, fat biopsies in postmenopausal women paralleled intake of dietary PUFAs from the semiquantitative Harvard-Willett Diet Assessment Questionnaire (31) used in the present study.

Observational studies of diet-disease associations cannot address lifetime dietary intake or dietary change and cannot completely exclude confounding if higher intakes of n–3 fatty acids or a low ratio of n–6 to n–3 fatty acids covaries with a more healthy diet (eg, more calcium) or another healthy lifestyle pattern (eg, more exercise). In this study, calcium intake and physical activity were higher in individuals with more favorable PUFA ratios, but adjustment for these differences did not materially change the associations.

In conclusion, PUFAs in the diet appear to be a modifiable risk factor that may be related to the development of osteoporosis (32). Experts suggest that 2 concomitant changes need to occur in fatty acid consumption to reduce the risk of chronic disease: an increased intake of n–3–rich foods and a decrease in the consumption of animal and vegetable foods that contain large amounts of n–6 fatty acids (3, 4). Randomized clinical trials are needed to test whether fatty acids have an effect on bone. If fatty acid supplementation is effective, it could offer a safe, relatively inexpensive approach to the prevention of osteoporosis.

LAW, EB-C, and DvM were responsible for the study concept and design, acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, and statistical analysis. LAW was responsible for drafting the manuscript. EB-C obtained the funding for the study. None of the authors had any conflicts of interest in connection with this study.

REFERENCES