

Fatty acid consumption and risk of fracture in the Women's Health Initiative¹⁻⁴

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ABSTRACT

Background: Fatty acids (FAs) may be important dietary components that modulate osteoporotic fracture risk.

Objective: The objective was to examine FA intake in relation to osteoporotic fractures.

Design: The participants were postmenopausal women enrolled in the Women's Health Initiative ($n = 137,486$). Total fractures were identified by self-report; hip fractures were confirmed by medical record review. FA intake was estimated from baseline food-frequency questionnaires and standardized to total caloric intake. No data on omega-3 ($n-3$) FA supplements were available. Cox proportional hazard models were constructed to estimate risk of fracture.

Results: Higher saturated FA consumption was associated with higher hip fracture risk [quartile 4 multivariate-adjusted hazard ratio (HR): 1.31; 95% CI: 1.11, 1.55; P for trend = 0.001]. Lower total fracture risk was associated with a higher monounsaturated FA intake (quartile 3 HR: 0.94; 95% CI: 0.89, 0.98; P for trend = 0.050) and polyunsaturated FA intake (quartile 4 HR: 0.95; 95% CI: 0.90, 0.99; P for trend = 0.019). Unexpectedly, higher consumption of marine $n-3$ FAs was associated with greater total fracture risk (quartile 4 HR: 1.07; 95% CI: 1.02, 1.12; P for trend = 0.010), whereas a higher $n-6$ FA intake was associated with a lower total fracture risk (quartile 4 HR: 0.94; 95% CI: 0.89, 0.98; P for trend 0.009).

Conclusions: These results suggest that saturated FA intake may significantly increase hip fracture risk, whereas monounsaturated and polyunsaturated FA intakes may decrease total fracture risk. In postmenopausal women with a low intake of marine $n-3$ FAs, a higher intake of $n-6$ FAs may modestly decrease total fracture risk. This trial was registered at clinicaltrials.gov as NCT00000611. *Am J Clin Nutr* 2010;92:1452-60.

INTRODUCTION

Osteoporosis is a major public health concern that disproportionately affects women (1). Each year in the United States, there are >1.5 million fractures attributable to osteoporosis, including 329,000 hip fractures (2). Osteoporosis results in a lifetime fracture risk of nearly 40% for women (3). It is therefore vital to identify common exposures that affect this pervasive public health problem.

Nutrition is an environmental exposure that is important to skeletal health. A large number of nutritional factors ranging from protein intake to minerals and vitamins have been identified

as playing a potential role in modifying the risk of osteoporosis (4-9). Nutrients may act directly by modifying bone turnover, stimulating bone formation, or affecting calcium balance and may ultimately modify bone mineral density (BMD), mineralization, bone quality, and fracture risk. Research investigating intakes of saturated, monounsaturated, and polyunsaturated fatty acids (FAs) in relation to BMD and fracture risk has yielded varied results (10, 11).

Recently, the essential FAs of the omega-6 ($n-6$) and omega-3 ($n-3$) families have been suggested to play a role in bone health and metabolism (12-20). In most studies, $n-3$ FAs reduce and $n-6$ FAs increase markers of bone turnover in animal (18, 20, 21) and cell models (22, 23), possibly related to the more anti-inflammatory properties associated with the family of $n-3$ FAs. In humans, higher consumption of $n-3$ FAs and lower $n-6:n-3$ ratios have been associated with higher BMD (13-15)

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and more favorable bone turnover markers (12). In prospective cohort studies, greater dietary intake of n-3 FAs is associated with lower concentrations of circulating inflammatory cytokines (24), whereas greater consumption of n-6 FAs have been associated with increased fracture risk (11). Data from fish-oil supplementation studies suggest that there may be an optimum level of dietary eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) needed to promote the most favorable conditions for bone remodeling (25). The purpose of the current study was to examine dietary fat intake, specifically n-3 and n-6 FAs, the type of n-3 FA and the n-6:n-3 FA ratio, and risk of fractures in postmenopausal women who were enrolled in the Women's Health Initiative (WHI) studies. The primary hypothesis was that n-3 FAs would reduce the risk of osteoporotic fractures. Secondary hypotheses were that higher n-3 FA consumption from both marine and nonmarine sources would be associated with lower fracture risk, and higher intake of n-6 FAs as well as a higher n-6:n-3 FA ratio would be associated with increased fracture risk.

SUBJECTS AND METHODS

Study population

The WHI is the largest study to date focusing on prevention and control of some of the most common diseases contributing to morbidity and mortality in postmenopausal women, including coronary artery disease, breast and colorectal cancers, and osteoporotic fractures. Details of the study design and methods were previously reported (26). Between 1993 and 1998, ethnically diverse women ($n = 161,808$) between 50 and 79 y of age enrolled in the WHI. A total of 68,132 women joined ≥ 1 of the 3 randomized clinical trials; a low fat dietary modification trial (DM) compared with usual diet, 2 placebo-controlled hormone therapy (HT) trials with estrogen alone or estrogen plus progestin and a calcium plus vitamin D supplement trial compared with placebo (CaD). Participants not eligible or not interested in the clinical trials were able to enroll in the Observational Study (OS); this included 93,676 women. The study was reviewed and approved by the Human Subjects Review Committee at each of the 40 clinical centers nationwide, and all participants provided signed informed consent.

The study population for this analysis included women for whom individual FA intake data were available at enrollment in the WHI OS and clinical trial cohorts, excluding DM intervention arm participants ($n = 137,486$). Information on demographics, health and medication history, and lifestyle factors was collected by using screening questionnaires and interviews at baseline enrollment.

Dietary assessment

Dietary intake was assessed by using a semiquantitative food-frequency questionnaire (FFQ) adapted from instruments used in previous clinical trials. The FFQ contained 122 questions on food items or food groups, 19 adjustment questions designed to allow more precise analysis of fat intake, and 4 summary questions addressing usual intake of fruit, vegetables, and added fats (27). Nutrient intakes were obtained from the FFQ by using a database derived from the University of Minnesota's Nutrition Coordinating

Center (version 30; Minnesota Nutrition Data System for Research, Minneapolis, MN) (28). Whereas WHI collected data on dietary supplement use, the protocol did not include the collection of data on either n-3 or n-6 supplements; therefore, information on FA supplements was not available for inclusion in these analyses.

Fracture ascertainment

Fracture outcomes were self-reported by participants on a health update questionnaire. For women in the clinical trials, these questionnaires were administered every 6 mo either at clinic visit or by mail or phone. OS participants completed the health updates by mail or phone annually. Proxy interviews regarding health outcomes were conducted for those women who were unable or deceased (6). Total fractures were defined as all clinical fractures with the exception of fractures of the fingers and toes, ribs, sternum/chest, skull/face, and cervical vertebrae. Fractures were based on self-report in the OS cohort with the exception of hip fractures, which were centrally adjudicated by trained and blinded physicians in both the clinical trials and OS. Medical records (radiology, surgery reports) were requested for all fractures reported in the clinical trials, and these were centrally adjudicated by a physician. There was 96% agreement between central and local adjudication of hip fractures (29). In addition, 71% of self-reported single-site fractures could be confirmed in a subset analysis of WHI participants from the 3 clinical centers that fully adjudicated all fractures (30).

Statistical analyses

FA intake was determined by using the calculated values of total fat, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), α -linolenic acid (ALA), EPA + DHA, total n-3 FAs, linoleic acid, arachidonic acid, total n-6 FAs, and the n-6:n-3 FA ratio from the dietary information gathered by using the baseline FFQ. FA intake was standardized to total caloric intake (g/1000 kcal) to account for variations in energy consumption. The FA intakes were categorized based on quartiles of the FA consumption in the study population.

HT use was defined as "yes" for women randomly assigned to the estrogen-alone or estrogen + progestin arms of the clinical trials and for OS women reporting current HT use at baseline. HT use was defined as "no" for women randomly assigned to placebo and for OS women reporting past use or never use of HT at baseline. Covariates were identified as potential confounders based on multivariate models of fracture risk in the WHI (31, 32). Primary outcome variables were hip fractures and total fractures.

Descriptive statistics were calculated for covariates of interest and dietary intake variables by total n-3 FA intake in quartiles. General linear model *t* tests for slope were performed to compare the means of continuous covariates by total n-3 FA intake in quartiles. Chi-square tests of association and chi-square tests for trend were performed to compare the quartiles of total n-3 FA intake by the categorical variables. Age-adjusted fracture rates according to FA intake in quartiles were computed.

Cox proportional hazards modeling was performed to estimate the risk of fracture by total fat, SFA, MUFA, PUFA, n-3, and n-6 FA intakes. We originally ran the analysis with the

predictor variable (FA intake) expressed in 2 ways: 1) FA intake in grams with total energy intake included as a covariate in the model, and 2) FA intake as g/1000 kcal. Both approaches yielded similar results. Because fat recommendations are generally reported as % of energy, we chose to report our results in this format. Hazard ratios (HRs) with 95% CIs were calculated to examine the relation between fracture risk and baseline FA intake in quartiles and categories of n-6:n-3 ratio. A trend test across the quartiles of FA intake and the n-6:n-3 ratio was preformed for all models. Cox proportional hazards modeling was performed to estimate the risk of fracture by HT use and the n-6:n-3 FA ratio and by calcium intake and the n-6:n-3 FA ratio. Likelihood ratio tests were performed to test for interactions between HT use and the n-6:n-3 FA ratio and between calcium intake and the n-6:n-3 FA ratio.

Two sets of Cox models were constructed. The first, simpler model was adjusted only for age and race-ethnicity. Covariates for the second, full model were chosen a priori based on previous research of fracture risk in WHI participants (31, 32) and included age, race-ethnicity, education, marital status, family history of fracture, fracture after age 54 y, number of falls in the past 12 mo, height, weight, total vitamin D intake (food plus supplements), HT use, antianxiety or antidepressant medication use, bisphosphonate use, corticosteroid use, smoking status, arthritis, depression [Center for Epidemiologic Studies–Depression Scale (CES-D)], general health, parity, treated diabetes, and weekly exercise [metabolic equivalents (METs) per week]. Because of the large significant difference in calcium intake between women in different quartiles of n-3 FA intake, we added calcium intake (food plus supplements) as a covariate in the full Cox models. FA intake was standardized to total energy intake in all models, with the exception of models investigating the n-6:n-3 FA ratio. In these models, total energy intake was added as a covariate because this calculated ratio was based on FA intake in grams and not standardized to total caloric intake.

To investigate the public health significance of our results, we estimated population attributable risk (PAR) as follows:

$$\text{PAR} = p(\text{HR} - 1) / 1 + p(\text{HR} - 1) \quad (1)$$

where p is the prevalence of the risk factor. We included established risk factors for fracture (lowest quartile of body weight, parental history of fracture, current smoking, and current corticosteroid use) for comparison as well as types of FAs that we found were significantly associated with increased hip fracture and total fracture risk. All analyses were carried out by using SAS version 9.1 (SAS Institute Inc, Cary, NC).

RESULTS

Descriptive data

Data on FA intake were available for 137,486 women from the WHI who were administered an FFQ at the baseline visit. Approximately 38% of these women were on HT at the start of WHI, and 9.5% were randomly assigned to receive calcium and vitamin D treatment in the CaD trial. Characteristics of the study population as a whole and by quartiles of n-3 FA intake are reported in **Table 1**. Mean self-reported energy intake of participants was 1622 kcal/d. Total daily calcium and vitamin D intakes from the

diet and supplements averaged 1199 mg/d and 375 IU/d, respectively, with the highest consumption of both micronutrients observed in the lowest quartile of n-3 FA intake. Total fat intake averaged 32% of energy; SFAs and MUFAs provided 10.6% and 12.1% of total energy, respectively. Total PUFA consumption supplied an average of 6.7% of energy, primarily from n-6 FAs (5.8% of energy). The vast majority of women (97%) reported consuming $\leq 10\%$ of energy from n-6 FAs. Total n-3 FA intake averaged 0.78% of energy (range: 0.08–5.64% of energy). Nonmarine sources (ALA) accounted for most of the n-3 FAs consumed (0.70% of energy), whereas marine sources (EPA + DHA) supplied only 0.07% of energy. The mean ratio of n-6:n-3 FA in this cohort was 7.78. Over an average follow-up of 7.8 y, 20,399 total fractures were reported, including 1638 hip fractures.

Dietary fat and risk of hip and total fractures

After multivariate adjustment and standardization of fat intake to energy intake, higher SFA consumption was significantly associated with higher hip fracture risk (quartile 4 HR: 1.31; 95% CI: 1.11, 1.55; P for trend 0.001) (**Table 2**). A higher intake of MUFAs was significantly associated with slightly lower total fracture risk (quartile 3 HR: 0.94; 95% CI: 0.89, 0.98; P for trend = 0.050), as was a higher intake of PUFAs (quartile 4 HR: 0.95; 95% CI: 0.90, 0.99; P for trend = 0.019).

n-3 Fatty acids and risk of hip and total fractures

Age-adjusted total fracture rates (per 1000 person-years) by quartiles of total n-3 FA intake (% of energy) decreased with increasing n-3 FA intake (quartile 1: 21.53; quartile 4: 19.71). Whereas the lowest hip fracture rates were also associated with the highest n-3 FA intake, there was no linear relation noted between total n-3 FA and hip fractures; the highest hip fracture rates were seen in the second quartile of intake (quartile 2: 1.69; quartile 4: 1.51).

Multivariate HRs for risk of hip and total fractures according to individual PUFA intake and total n-3 and n-6 FA intakes are reported in **Table 3**. Although a higher consumption of EPA + DHA was associated with lower hip fracture risk in the model adjusted only for age and race-ethnicity (quartile 4 HR: 0.80; 95% CI: 0.70, 0.92; P for trend = 0.004), this association was not significant when all covariates were included in the model. No significant relation was noted with total n-3 FA intake or ALA intake and total fracture risk in the fully adjusted model. In contrast with the inverse direction of association noted with consumption of EPA+DHA and hip fracture, women with higher intakes of EPA + DHA had a modest increase in risk of total fractures after multivariate adjustment (quartile 4 HR: 1.07; 95% CI 1.02, 1.12; P for trend = 0.01).

n-6 Fatty acids and risk of hip and total fractures

The relation between total n-6 FA intake and age-adjusted total fracture rates was similar to that noted with n-3 FA intake, with a decrease in total fracture rates in individuals consuming the highest n-6 FA (quartile 1: 21.91; quartile 4: 19.58); however, no relation was seen between rates of hip fracture and total n-6 FA intake (data not shown). Likewise, no significant associations were noted with n-6 FA intake and hip fracture risk

TABLE 1

Baseline characteristics of Women's Health Initiative (WHI) observational and clinical trial participants by quartile (Q) of total n-3 fatty acid (FA) intake¹

Characteristics	WHI cohort	Total n-3 FA intake				P value
		Q1 (<0.581% of energy)	Q2 (0.581–0.736% of energy)	Q3 (0.737–0.932% of energy)	Q4 (>0.932% of energy)	
No. of subjects	137,486	34,345	34,492	34,213	34,436	
Age at screening (y)	63 ± 7 ²	63 ± 7	63 ± 7	63 ± 7	63 ± 7	0.0026
Race-ethnicity [n (%)]						
White	114,808 (83.5)	30,583 (89.0)	29,623 (85.9)	28,182 (82.4)	26,420 (76.7)	<0.0001
Black	11,398 (8.3)	1580 (4.6)	2462 (7.1)	3200 (9.4)	4156 (12.1)	
Hispanic	5250 (3.8)	1248 (3.6)	1222 (3.5)	1297 (3.8)	1483 (4.3)	
American Indian	575 (0.4)	141 (0.4)	131 (0.4)	158 (0.5)	145 (0.4)	
Asian/Pacific Islander	3558 (2.6)	359 (1.0)	624 (1.8)	918 (2.7)	1657 (4.8)	
Unknown	1897 (1.4)	434 (1.3)	430 (1.2)	458 (1.3)	575 (1.7)	
Education, college degree, or higher [n (%)] ³⁻⁵	54,807 (40.2)	14,172 (41.6)	13,706 (40.0)	13,456 (39.6)	13,473 (39.4)	<0.0001
Parental fracture [n (%)] ⁴⁻⁷	50,649 (36.8)	13,058 (38.0)	12,958 (37.6)	12,514 (36.6)	12,119 (35.2)	<0.0001
Fracture on or after age 55 y [n (%)]	17,614 (12.8)	4472 (13.0)	4498 (13.0)	4359 (12.7)	4285 (12.4)	0.0109
≥Two falls in the past 12 mo [n (%)] ⁵⁻⁷	16,626 (12.1)	4306 (12.5)	4324 (12.5)	4149 (12.1)	3847 (11.2)	<0.0001
Treated diabetes [n (%)] ³⁻⁷	5958 (4.3)	1083 (3.2)	1476 (4.3)	1603 (4.7)	1796 (5.2)	<0.0001
Current HT user [n (%)] ⁵⁻⁷	54,716 (39.8)	13,768 (40.1)	13,920 (40.4)	13,655 (39.9)	13,373 (38.8)	0.0004
Current smoker [n (%)]	9425 (6.9)	1928 (5.6)	2197 (6.4)	2439 (7.1)	2861 (8.3)	<0.0001
Arthritis [n (%)]	65,168 (47.4)	16,342 (47.6)	16,477 (47.8)	16,177 (47.3)	16,172 (47.0)	0.0953
Very good to excellent general health [n (%)] ^{3-6,8}	79,730 (58.0)	21,198 (61.7)	20,025 (58.1)	19,370 (56.6)	19,137 (55.6)	<0.0001
Depression (CES-D score)	0.04 ± 0.13	0.04 ± 0.13	0.04 ± 0.13	0.04 ± 0.13	0.04 ± 0.13	0.3397
BMI (kg/m ²) ^{3-6,8}	27.8 ± 5.90	27.2 ± 5.62	27.9 ± 5.90	28.1 ± 6.03	28 ± 6.09	<0.0001
Physical activity (METs/wk) ^{3-6,8}	12.8 ± 13.93	14.3 ± 14.78	12.7 ± 13.59	12.1 ± 13.44	12.1 ± 13.75	<0.0001
Energy intake (kcal/d)	1622 ± 633	1580 ± 587	1645 ± 629	1660 ± 657	1603 ± 653	<0.0001
Protein intake (g/d) ^{3-5,7,8}	68 ± 28.47	67 ± 27.76	70 ± 28.73	69 ± 29.09	65 ± 28.02	<0.0001
Fat intake (g/d)	59 ± 32.13	47 ± 25.14	58 ± 29.53	64 ± 32.89	68 ± 35.85	<0.0001
Saturated fat	20 ± 11.59	17 ± 9.92	20 ± 11.31	21 ± 12.18	21 ± 12.2	<0.0001
Monounsaturated fat	22 ± 12.46	18 ± 9.84	22 ± 11.51	24 ± 12.75	26 ± 13.83	<0.0001
Polyunsaturated fat	12 ± 6.89	9 ± 4.327	11 ± 5.333	13 ± 6.392	16 ± 8.51	<0.0001
n-6 FAs	10.7 ± 6.16	7.6 ± 3.991	9.8 ± 4.86	11.5 ± 5.774	13.9 ± 7.624	<0.0001
n-3 FAs	1.4 ± 0.764	0.8 ± 0.350	1.2 ± 0.47	1.5 ± 0.614	2.1 ± 0.901	<0.0001
ALA	1.27 ± 0.72	0.75 ± 0.33	1.07 ± 0.45	1.37 ± 0.59	1.87 ± 0.87	<0.0001
EPA + DHA	0.13 ± 0.12	0.08 ± 0.07	0.12 ± 0.09	0.14 ± 0.12	0.17 ± 0.17	<0.0001
Calcium intake (mg/d) ⁹	1199 ± 739.36	1358 ± 807.17	1235 ± 702.76	1155 ± 673.6	1048 ± 732.23	<0.0001
Vitamin D intake (IU/d) ⁹	375 ± 280.55	407 ± 292.05	381 ± 281.72	368 ± 274.99	345 ± 269.32	<0.0001

¹ All variables were assessed at the baseline screening visit. Dietary variables were assessed with a food-frequency questionnaire. CES-D, Center for Epidemiologic Studies-Depression Scale; HT, hormone therapy; METs, metabolic equivalents; ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. P values for categorical variables, except race-ethnicity, were obtained by using chi-square tests for linear trend. The P value for race was obtained by using a chi-square test for association. P values for continuous variables were obtained by using general linear model t tests for slope. Differences between all quartiles were found for race-ethnicity, current smoker, general health, energy, fat, saturated fat, monounsaturated fat, polyunsaturated fat, n-6 fatty acid, n-3 fatty acid, ALA, EPA + DHA, calcium, and vitamin D intakes (P < 0.0001).

² Mean ± SD (all such values).

³ Significant differences between Q1 and Q2, P < 0.0083.

⁴ Significant differences between Q1 and Q3, P < 0.0083.

⁵ Significant differences between Q1 and Q4, P < 0.0083.

⁶ Significant differences between Q2 and Q4, P < 0.0083.

⁷ Significant differences between Q3 and Q4, P < 0.0083.

⁸ Significant differences between Q2 and Q3, P < 0.0083.

⁹ Calcium and vitamin D values include the combined intake from food and supplements.

(Table 3). However, women in the highest quartile of total n-6 FA intake had a significant decrease in total fracture risk (quartile 4 HR: 0.94; 95% CI: 0.89, 0.98; P for trend = 0.009) compared with women in the lowest quartile of n-6 FA intake. Results for the most abundant dietary n-6 FA (linoleic acid) mirrored total n-6 FA results, whereas arachidonic acid intake did not substantially contribute to n-6 FA consumption (data not shown).

n-6:n-3 Ratio and risk of hip and total fractures

Cox proportional hazard models were fit to estimate the risk of hip and total fractures based on n-6:n-3 quartiles (Table 4). No significant relations associated with the ratio of dietary n-6:n-3 FA and hip fracture were observed, but a significant trend (P = 0.001) for lower total fracture risk was observed with n-6:n-3 FA ratios >6.43. An analysis of interactions between the

TABLE 2Multivariate hazard ratios (HRs) for risk of hip and total fracture by quartile (Q) of total dietary fat intake¹

Dietary fat	No. of subjects	Hip fracture				Total fracture			
		HR (95% CI) ²	<i>P</i>	HR (95% CI) ³	<i>P</i>	HR (95% CI) ²	<i>P</i>	HR (95% CI) ³	<i>P</i>
Total fat									
Q1 (3.89–25.97% of energy)	34,231	1.00	0.005	1.00	0.325	1.00	0.001	1.00	0.146
Q2 (25.98–32.24% of energy)	34,240	1.04 (0.91, 1.20)		1.01 (0.86, 1.19)		0.97 (0.93, 1.00)		0.99 (0.94, 1.03)	
Q3 (32.25–37.87% of energy)	34,211	1.09 (0.95, 1.25)		0.97 (0.82, 1.15)		0.93 (0.89, 0.96)		0.97 (0.92, 1.01)	
Q4 (37.88–51.35% of energy) ⁴	34,166	1.21 (1.06, 1.39)		1.11 (0.94, 1.32)		0.95 (0.91, 0.98)		0.97 (0.92, 1.02)	
SFA									
Q1 (1.25–8.28% of energy)	34,224	1.00	<0.001	1.00	0.001	1.00	0.135	1.00	0.873
Q2 (8.29–10.52% of energy)	34,227	1.05 (0.91, 1.22)		1.04 (0.88, 1.22)		0.96 (0.93, 1.00)		0.97 (0.93, 1.02)	
Q3 (10.53–12.77% of energy)	34,215	1.20 (1.04, 1.38)		1.12 (0.94, 1.32)		0.96 (0.92, 1.00)		0.99 (0.94, 1.04)	
Q4 (12.78–36.70% of energy)	34,182	1.34 (1.17, 1.53)		1.31 (1.11, 1.55)		0.97 (0.93, 1.01)		0.99 (0.94, 1.04)	
MUFA									
Q1 (1.03–9.63% of energy)	34,245	1.00	0.069	1.00	0.758	1.00	<0.001	1.00	0.050
Q2 (9.64–12.17% of energy)	34,215	1.11 (0.97, 1.28)		1.04 (0.89, 1.22)		0.98 (0.95, 1.02)		1.00 (0.95, 1.04)	
Q3 (12.18–14.51% of energy)	34,229	1.12 (0.98, 1.29)		1.04 (0.88, 1.22)		0.91 (0.88, 0.95)		0.94 (0.89, 0.98)	
Q4 (14.52–48.50% of energy)	34,159	1.14 (0.99, 1.32)		1.03 (0.87, 1.22)		0.95 (0.91, 0.98)		0.97 (0.92, 1.02)	
PUFA									
Q1 (0.71–5.16% of energy)	34,241	1.00	0.574	1.00	0.266	1.00	<0.001	1.00	0.019
Q2 (5.17–6.42% of energy)	34,212	0.99 (0.86, 1.13)		0.95 (0.81, 1.11)		0.99 (0.95, 1.03)		0.98 (0.94, 1.03)	
Q3 (6.43–7.89% of energy)	34,218	1.01 (0.88, 1.16)		0.91 (0.77, 1.07)		0.95 (0.91, 0.99)		0.96 (0.92, 1.01)	
Q4 (7.90–31.84% of energy)	34,177	1.03 (0.90, 1.19)		0.92 (0.78, 1.09)		0.93 (0.90, 0.97)		0.95 (0.90, 0.99)	

¹ HRs and 95% CIs were obtained from Cox proportional hazard models. *P* values are from tests for linear trend. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

² Models were adjusted for age and ethnicity.

³ Models were adjusted for age, ethnicity, education, marital status, family history of fracture, fracture on or after age 55 y, number of falls in past year, height, weight, total vitamin D intake, hormone therapy history, antianxiety or antidepressant medication use, bisphosphonate use, corticosteroid use, smoking status, arthritis, depression, general health assessment, parity, treated diabetes, weekly exercise, and total calcium intake.

⁴ The maximum total dietary fat intake was 100% of energy, but the 99th percentile was 51%.

n-6:n-3 ratio and calcium intake or HT did not modify the results.

Population attributable risk

Results of the analyses estimating PAR for types of FAs significantly associated with fractures are shown in **Table 5**. The PAR for the highest quartile of SFA intake for hip fracture was 5.5%, which was similar to the PAR for current smoking (5.6%), greater than the PAR for current corticosteroid use (1.5%), but less than the PAR for lowest quartile of body weight (8.3%) and parental history of fracture (7.3%).

The PAR for the lowest quartile of PUFA intake for total fractures was 1.1%, whereas both the lowest quartile of MUFA intake and the lowest quartile of n-6 FA intake had a PAR of 1.4%. By comparison, the PAR for current smoking for total fractures was 0.8% and current corticosteroid use was 0.7%, whereas the lowest quartile of body weight was not associated with a significant PAR for total fractures, and parental history of fracture was associated with a PAR of 8.1%. Because of the very narrow range of EPA + DHA intakes (quartile 4 represented only a 0.09-g increase in intake compared with quartile 1), the PAR for the highest EPA + DHA intake was not calculated.

DISCUSSION

This was the first study to comprehensively examine the relation of hip fracture and total fracture in postmenopausal women across categories of fat intake. In agreement with data from the

WHI Dietary Modification Trial (4), total fat intake was not associated with risk of hip or total fractures. However, in our study, a higher intake of SFA increased the risk of hip fracture in a dose-dependent manner. Data on fat consumption and risk of hip fracture are limited, but BMD data are widely available and frequently used as a marker to predict hip fracture risk (33). Our results support those of a recent analysis within the third National Health and Nutrition Examination Survey (NHANES III), which showed that a higher SFA intake was associated with lower BMD at the hip (10). In contrast with a case-control study of hospitalized individuals aged >65 y in Spain suggesting increased fracture risk with higher PUFA intakes (11), women in the WHI had a modestly lower total fracture risk with higher consumption of both MUFAs and PUFAs.

Consistent with previous research (11), total n-3 FA intake in WHI women was not associated with hip fracture or total fracture risk. Although research on n-3 FA intake and osteoporosis has focused on the marine-derived n-3 FAs EPA and DHA, we further explored risk of fractures in relation to intake of ALA—the predominant nonmarine n-3 FA in the US diet. Although we found no association with ALA consumption and hip or total fracture risk in our population, who had moderate ALA intake, prior research suggests that a diet high in ALA may reduce bone resorption in humans (12).

In our cohort, as in a recent report from the Cardiovascular Health Study (34), intake of EPA + DHA was not associated with hip fracture risk. However, consumption of these n-3 FAs was associated with a slightly higher total fracture risk in our study.

TABLE 3Multivariate hazard ratios (HRs) for risk of hip and total fractures by quartile (Q) of dietary polyunsaturated fatty acid (PUFA) intake¹

PUFAs	No. of subjects	Hip fracture				Total fracture			
		HR (95% CI) ²	<i>P</i>	HR (95% CI) ³	<i>P</i>	HR (95% CI) ²	<i>P</i>	HR (95% CI) ³	<i>P</i>
n-3 Fatty acids									
Total n-3									
Q1 (0.08, 0.58% of energy)	34,203	1.00	0.747	1.00	0.826	1.00	0.021	1.00	0.260
Q2 (0.58, 0.74% of energy)	34,348	1.11 (0.97, 1.27)		1.10 (0.94, 1.29)		1.01 (0.97, 1.05)		1.01 (0.97, 1.06)	
Q3 (0.74, 0.93% of energy)	34,070	1.03 (0.90, 1.18)		1.04 (0.89, 1.22)		0.97 (0.94, 1.01)		1.00 (0.95, 1.05)	
Q4 (0.93, 5.64% of energy)	34,227	1.05 (0.91, 1.21)		1.00 (0.84, 1.18)		0.96 (0.93, 1.00)		0.97 (0.93, 1.02)	
ALA									
Q1 (0.08, 0.51% of energy)	34,232	1.00	0.177	1.00	0.565	1.00	0.006	1.00	0.257
Q2 (0.51, 0.65% of energy)	34,193	1.03 (0.90, 1.19)		1.00 (0.85, 1.18)		1.02 (0.98, 1.06)		1.02 (0.97, 1.07)	
Q3 (0.65, 0.84% of energy)	34,298	1.09 (0.95, 1.25)		1.07 (0.91, 1.25)		0.98 (0.94, 1.02)		1.00 (0.96, 1.05)	
Q4 (0.84, 5.61% of energy)	34,125	1.08 (0.94, 1.24)		1.03 (0.87, 1.21)		0.95 (0.92, 0.99)		0.98 (0.93, 1.02)	
EPA + DHA									
Q1 (0.0, 0.03% of energy)	33,531	1.00	0.004	1.00	0.133	1.00	0.006	1.00	0.010
Q2 (0.03, 0.05% of energy)	35,218	0.90 (0.79, 1.03)		0.91 (0.78, 1.06)		1.01 (0.97, 1.05)		1.04 (0.99, 1.09)	
Q3 (0.05, 0.09% of energy)	33,619	0.91 (0.79, 1.04)		0.96 (0.82, 1.13)		1.03 (0.99, 1.07)		1.04 (0.99, 1.09)	
Q4 (0.09, 1.6% of energy)	34,480	0.80 (0.70, 0.92)		0.86 (0.72, 1.01)		1.05 (1.01, 1.10)		1.07 (1.02, 1.12)	
n-6 Fatty acids									
Total n-6									
Q1 (0.61, 4.47% of energy)	34,250	1.00	0.385	1.00	0.382	1.00	<0.001	1.00	0.009
Q2 (4.47, 5.61% of energy)	34,214	0.94 (0.81, 1.07)		0.90 (0.77, 1.06)		0.98 (0.94, 1.01)		0.98 (0.93, 1.02)	
Q3 (5.61, 6.94% of energy)	34,200	1.05 (0.92, 1.20)		0.94 (0.80, 1.10)		0.94 (0.91, 0.98)		0.96 (0.92, 1.01)	
Q4 (6.94, 28.33% of energy)	34,184	1.03 (0.90, 1.18)		0.91 (0.77, 1.08)		0.92 (0.89, 0.96)		0.94 (0.89, 0.98)	

¹ ALA, α -linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid. HRs and 95% CIs were obtained from Cox proportional hazard models. *P* values are from tests for linear trend.

² Models were adjusted for age and ethnicity.

³ Models were adjusted for age, ethnicity, education, marital status, family history of fracture, fracture on or after age 55 y, number of falls in past year, height, weight, total vitamin D intake, hormone therapy history, antianxiety or antidepressant medication use, bisphosphonate use, corticosteroid use, smoking status, arthritis, depression, general health assessment, parity, treated diabetes, weekly exercise, and total calcium intake.

One explanation for this unexpected positive correlation may be that the very low consumption of marine sources of n-3 FAs coupled with the lack of data on n-3 FA supplement use by women in the WHI affected our results. It is possible that any benefit of marine sources of n-3 FAs to fracture risk may require a threshold of intake not reached by our cohort, because the mean EPA + DHA consumption was 0.13 g/d, considerably less than the \approx 0.5 g/d minimally recommended by many global organizations (35). There was little variation in the range of EPA + DHA consumed by our cohort, which made it difficult to examine associations with higher marine n-3 FA intakes and

fracture. Interestingly, women who consumed the most EPA + DHA consumed the least calcium and vitamin D.

In comparison with other studies that examined n-6 FA consumption and reported no association (34) or an increased risk of fractures (11), our cohort consumed a smaller amount of n-6 FAs. For example, individuals in a Spanish study who consumed \geq 18 g n-6 FAs/d had a significantly elevated risk of fractures (11). However, WHI participants in the highest quartile of n-6 FA intake averaged only 13.9 g/d and had a 6% decrease in relative risk of total fractures. Also of note, men and women in the Spanish cohort reported consuming \approx 30% more energy,

TABLE 4Multivariate hazard ratios (HRs) for risk of hip and total fractures based on dietary n-6:n-3¹

n-6:n-3 Quartile	No. of subjects	Hip fracture				Total fracture			
		HR (95% CI) ²	<i>P</i>	HR (95% CI) ³	<i>P</i>	HR (95% CI) ²	<i>P</i>	HR (95% CI) ³	<i>P</i>
1 (0.49, 6.433)	34,199	1.00	0.180	1.00	0.726	1.00	<0.001	1.00	0.001
2 (6.434, 7.664)	34,225	0.94 (0.82, 1.08)		0.93 (0.79, 1.09)		0.92 (0.88, 0.96)		0.93 (0.89, 0.98)	
3 (7.665, 8.898)	34,216	1.09 (0.95, 1.25)		1.04 (0.89, 1.23)		0.91 (0.88, 0.95)		0.92 (0.88, 0.97)	
4 (8.900, 39.01) ⁴	34,208	1.05 (0.92, 1.21)		0.93 (0.79, 1.10)		0.92 (0.89, 0.96)		0.92 (0.88, 0.97)	

¹ HRs and 95% CIs were obtained from Cox proportional hazard models. *P* values are from tests for linear trend.

² Models were adjusted for age, ethnicity, and total energy intake.

³ Models were adjusted for age, ethnicity, total energy intake, education, marital status, family history of fracture, fracture on or after age 55 y, number of falls in past year, height, weight, total vitamin D intake, hormone therapy history, antianxiety or antidepressant medication use, bisphosphonate use, corticosteroid use, smoking status, arthritis, depression, general health assessment, parity, treated diabetes, weekly exercise, and total calcium intake.

⁴ The maximum n-6:n-3 ratio was 39.01, but the 99th percentile was 14.1.

TABLE 5
Hazard ratios (HRs) and population attributable risk (PAR) of fatty acid (FA) intake and fracture risk factors¹

Fracture type and exposure	Prevalence	HR (95% CI)	PAR ² (95% CI)
Hip fractures			
Highest (Q4) SFA intake	0.25	1.235 (1.108, 1.375)	0.055 (0.026, 0.086)
Lowest (Q1) body weight	0.25	1.361 (1.228, 1.508)	0.083 (0.054, 0.113)
Parental history of fracture	0.39	1.197 (1.081, 1.325)	0.073 (0.031, 0.115)
Current smoking	0.0695	1.849 (1.547, 2.209)	0.056 (0.037, 0.077)
Current corticosteroid use	0.0095	2.583 (1.908, 3.497)	0.015 (0.009, 0.023)
Total fractures			
Lowest (Q1) MUFA intake	0.25	1.056 (1.024, 1.089)	0.014 (0.006, 0.022)
Lowest (Q1) PUFA intake	0.25	1.045 (1.013, 1.078)	0.011 (0.003, 0.019)
Lowest (Q1) n-6 FA intake	0.25	1.055 (1.023, 1.089)	0.014 (0.006, 0.022)
Lowest (Q1) body weight	0.25	0.999 (0.967, 1.031)	0.000 (-0.008, 0.008)
Parental history of fracture	0.399	1.220 (1.185, 1.255)	0.081 (0.069, 0.093)
Current smoking	0.0695	1.110 (1.051, 1.173)	0.008 (0.003, 0.012)
Current corticosteroid use	0.0095	1.744 (1.557, 1.953)	0.007 (0.005, 0.009)

¹ Q, quartile; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. HRs and 95% CIs were obtained from Cox proportional hazard models. All models were adjusted for age and ethnicity.

² Calculated as follows: prevalence \times (HR - 1)/1 + prevalence \times (HR - 1).

including 37% more total fat, almost twice as much MUFAs, and nearly 10 times more n-3 FAs as did women in the WHI. These dramatic differences in fat intake, as well as dietary patterns with variations in nutrients that may modify bone remodeling such as vitamin D, vitamin K, magnesium and potassium, may have contributed to our contrasting results.

Mechanisms by which n-3 and n-6 FAs may affect fracture risk are not known, but inflammation may be a contributing factor. The n-6 FAs are precursors of both antiinflammatory (prostacyclin, lipoxin A₄, and epoxyeicosatrienoic acid) and proinflammatory eicosanoids [prostaglandin E₂ (PGE₂), thromboxane A₂, and leukotriene B₄]. PGE₂ is the predominant prostaglandin found in bone cells and a potent stimulator of bone resorption at high concentrations (36, 37). Tumor necrosis factor- α , a cytokine that may promote bone resorption, is stimulated by PGE₂ (12). EPA and DHA supplementation in the form of fish oil reduce tumor necrosis factor- α by 70-77% and PGE₂ by 28-55%, depending on the LA and ALA content of the diet (38). PGE₂ decreases osteoprotegerin production and increases receptor activator of nuclear transcription factor κ B ligand (RANKL) expression. This action lowers the osteoprotegerin:receptor activator of nuclear transcription factor- κ B ligand ratio, which is critical in the pathogenesis of resorptive bone disease (39). It has been suggested that the effect of PGE₂ on bone formation may be dose related: stimulatory at low levels and inhibitory at high levels (36). The optimal intake of n-3 and n-6 FAs for bone health is unknown; however, on the basis of results of this study, it is possible that current recommendations for n-6 FA intake in the range of 5-10% of total energy (40) may contribute to a favorable environment for lower fracture risk.

Women in our study with n-6:n-3 FA ratios >6.43 had a modest reduction in total fracture risk. This is in contrast with previous reports of either no relation of the n-6:n-3 ratio to fractures (11, 34) or a beneficial relation of lower n-6:n-3 ratios to higher BMD (14). International recommendations for n-6:n-3 FA ratios, based primarily on cardiovascular benefits, are between 4 and 7.5 (35). Our data suggest that an n-6:n-3

FA ratio between 6.43 and 7.66 is also associated with a 7% reduction in relative risk of total fractures, with a 1% additional decline in relative risk with higher ratios.

Strengths and limitations

The strengths of this study include the large sample size of ethnically and geographically diverse postmenopausal women, high follow-up rates over time, central adjudication of hip fracture outcomes, details on a large number of clinical risk factors for fracture, and an FFQ specifically designed to refine analysis of fat intake and include numerous nutritional covariates (27).

One limitation of this study was the lack of data on fish-oil or n-3 FA supplements. However, the use of n-3 supplements in the United States during the WHI data collection period was relatively small, \approx 7.5% according to the US FDA Health and Diet Survey of 2002 (41), and we would expect the use of n-3 supplements to perhaps be even lower in WHI women during the 1993-1998 baseline enrollment period. The reliability of nutrient intake data from the FFQ and the use of a single questionnaire (administered at baseline) were also limiting factors in this research, but the intake of essential fatty acids was shown to correlate well with plasma and erythrocyte concentrations (12, 38, 42), and inaccuracies in self-reported intake should have been evenly distributed throughout the women in our sample. Finally, because of the observational nature of this research and the unavailability of inflammatory markers or serum vitamin D concentrations, no causal or mechanistic relations can be determined.

Conclusions

In conclusion, a higher SFA intake was associated with higher hip fracture risk, whereas higher overall MUFA and PUFA intakes were associated with slightly lower total fracture risk in this cohort of postmenopausal women. No association of total n-3 FA or ALA intake to fracture risk, was observed, but a small increase in risk of total fractures was observed with

a greater intake of EPA + DHA. In addition, women who consumed more total n-6 FAs had a lower total fracture risk. An n-6:n-3 ratio >6.43 offered modest protection against total fractures in this sample of women with a moderate consumption of n-6 FAs and low intake of marine n-3 FAs. These results support current recommendations of 5–10% of total energy as n-6 FAs. Although a beneficial dose of long-chain FAs cannot be determined from this analysis, our results suggest that the type of FA consumed may play a role in a diet that decreases osteoporotic fracture risk.

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