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# An Investigation of the Association Between Omega 3 FA and Bone Mineral Density Among Older Adults: Results From the National Health and Nutrition Examination Survey Years 2005–2008

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## An investigation of the association between omega 3 FA and bone mineral density among older adults: results from the National Health and Nutrition Examination Survey years 2005–2008

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### Abstract

**Summary**—The relation of omega 3 fatty acids (n-3 FA) with bone mineral density (BMD) was assessed among adults >60 years; NHANES data (2005–2008). The association of dietary n-3 FA with measures of hip BMD was equivocal, but n-3 FA supplement use was significantly associated with higher spine BMD—a finding that deserves further study.

**Introduction**—Associations between polyunsaturated fatty acids and bone mineral density are not well understood.

**Purpose**—To evaluate the cross-sectional relation between dietary omega 3 fatty acid intake (specifically docosahexaenoic acid, eicosapentaenoic acid, and octadecatetraenoic) and BMD at the hip and spine among older adults.

**Methods**—Omega 3 FA intake (g/day) was assessed from two 24-h recalls using the National Health and Nutrition Examination Survey (NHANES, in 2005–2008); and omega 3 FA supplement use (yes/no) via questionnaire. Multivariable regression models were developed to explain variance in femoral neck, total femur, and lumbar spine BMD among 2,125 men and women over 60 years.

**Results**—Mean age was 70 years. In adjusted models, dietary omega 3 FA were marginally associated with greater femoral neck BMD ( $p = 0.0505$ ), but not with total femur BMD ( $p = 0.95$ )

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**Conflict of interests** None.

or lumbar spine BMD ( $p = 0.74$ ). Omega 3 supplement use was significantly positively associated with lumbar spine BMD ( $p = 0.005$ ) but not with femoral neck or total femur BMD.

**Conclusions**—Dietary intakes of omega 3 FA were marginally associated with femoral neck BMD; however, omega 3 supplement use was significantly associated with higher lumbar spine BMD in older adults. These results emphasize the need for assessment of total omega 3 intakes (diet and supplements) to provide a greater range of intake and a more accurate picture of the relation between omega 3 FA and BMD.

### Keywords

Bone mineral density; NHANES; Omega-3 fatty acids; Polyunsaturated fatty acids

## Introduction

Osteoporosis is a disease characterized by low bone mass and structural deterioration of bone tissue leading to bone fragility and an increased risk of fractures of the hip, spine, and wrist [1]. Hip fractures are most detrimental with 20 % increased mortality rates in the first year following a fracture [2], leading to a total expenditure of \$17 billion [3]. Nutritional regimens to prevent and treat osteoporosis are important. Dietary calcium and vitamin D are the focus of most nutrition studies, while the impact of other dietary components such as polyunsaturated fatty acids (PUFA) on bone health is less understood.

Through a series of elongation and desaturation steps, the parent omega 3 PUFA (n-3), alpha linolenic acid (ALA), is metabolized to two longer chain FA: docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Derivatives of EPA and DHA include various eicosanoids and docosanoids which have anti-inflammatory properties. The metabolism of n-3 FA occurs in tandem with that of the omega 6 PUFA (n-6) family where linoleic acid is metabolized to its predominant product arachidonic acid (AA). The typical American diet provides a ratio of n-6 to n-3 FA of ~8–12:1 [4]. However, the optimal ratio may be closer to 2–3:1 [5]. Epidemiological evidence suggests greater intakes of n-3 FA relative to n-6 are protective of bone [6] because n-3 FA possibly modulate inflammatory mediators during bone remodeling [7–9]. Recent research suggests these effects may be due to the long-chain n-3 FA, DHA, and EPA [10]. However, some evidence on the relation between dietary n-3 FA and BMD is conflicting and results differ based on population, sex, age, and primary bone outcome (i.e., bone mineral density, risk of fracture, or bone biomarkers) [11–17].

The present study is designed to evaluate the relation between dietary n-3 FA (specifically DHA, EPA, and stearidonic acid [SDA] as its biological effects mimic that of EPA [18]) and BMD using data from the National Health and Nutrition Examination Survey (NHANES). This study is unique in that it uses a diverse data set which is representative of the US population. To our knowledge no such study has been conducted using the latest national data from large numbers of older men and women in NHANES. We hypothesized that greater intakes of EPA, DHA, and SDA would be positively associated with BMD at the hip and spine among men and women over the age of 60 years.

## Subjects and methods

### Sample population and data collection

The NHANES is the only national survey that collects extensive health information from both face-to-face interviews and medical examinations. Its data provide a cross-sectional picture of health and nutrition in the US population. The survey uses a complex, stratified, multilevel, probability-cluster sampling design. A detailed description of the NHANES plans and procedures is provided elsewhere [19].

The National Center for Health Statistics (NCHS) collects NHANES data in biennial cycles. The analyses reported in this paper utilized data from the 2005–2006 and 2007–2008 cycles. Men and women aged 60 years and older were included in the study sample because they are at greatest risk for osteoporosis and fracture. Our data set, therefore, included 2,125 men and women with two complete dietary recalls, and had valid BMD scans of the hip and 1,445 had valid BMD scans of the spine. Written informed consent was obtained from all participants or proxies, and the survey protocol was approved by the Research Ethics Review Board of NCHS [20].

### Dietary interview and analysis

The objective of the dietary interview component is to recall all types of foods eaten and amounts consumed in a 24-h period of time. The dietary interview was conducted in person at a mobile examination center. All interviewers were trained and also were monitored during the interview process. Data were collected by NHANES interviewers using a US Department of Agriculture (USDA) dietary data collection instrument, the Automated Multiple Pass Method [21]. This method uses a five-step procedure to quantify 24-h food intake [22, 23]. The USDA's Food and Nutrient Database for Dietary Studies, Version 3.0 was used with the 2005–2006 data, and Version 4.1 was used for the 2007–2008 data. In the present study, only data on survey participants with dietary recall status codes of "reliable" were used. A code of reliable indicates that there were no missing reference values for any nutrient based on food items cited in the 24-h recall.

The NHANES oversamples low-income persons, adolescents 12–19 years, persons 60+ years of age, African Americans, and Mexican Americans. To account for this, sampling weights are provided to support estimation of unbiased summary statistics for the US population. Sampling weights specifically related to the sub-sample of survey participants who completed the 24-h dietary recall were used in all analyses.

Nutrient intake was calculated by averaging the 2 days of recall. Available nutrient variables in the NHANES representing dietary n-3 FA intake as assessed by 24-h recall include EPA, DHA, and SDA, which were added together as a total estimate of intake. SDA was included in the analyses as its biological properties have been shown to be similar to EPA [18, 24].

The remaining PUFA nutrient variables (octadecadienoic, octadecatrienoic, eicosatetraenoic, and docosapentaenoic) were added together to serve as a covariate representative of other dietary PUFA (a combination of other n-3 and n-6 FA). The n-3 FA alpha-linolenic and the n-6 FA gamma-linolenic are both isomers of octadecatrienoic acid. The variable in NHANES termed octadecatrienoic acid was not classified as alpha- or gamma-linolenic acid. Therefore, for the purposes of this paper, octadecatrienoic acid was assumed to be a combination of both the n-6 and n-3 FA. For that reason, the ratio of n3:n6 FA could not be confidently determined and was not included in analysis.

### BMD measurements

BMD ( $\text{g}/\text{cm}^2$ ) of the hip and spine was measured by dual-energy X-ray absorptiometry on all eligible participants. Only individuals noted in the NHANES database with valid BMD scans were used in statistical analyses. Both the femur and spine scans were performed with a Hologic QDR-4500A fan-beam densitometer (Hologic, Inc., Bedford, Massachusetts); Hologic Discovery software (v12.4) was used to analyze all femur BMD scans and Hologic APEX v3.0 software to analyze all spine BMD measurements. The left hip was routinely scanned. In the case of left hip fracture, pin, or replacement, the right hip was scanned. Rigorous quality control standards were routinely employed. Further detail can be found elsewhere [25].

## Covariate measurements

Covariates commonly suspected to affect bone density were grouped for statistical analyses. Demographic covariates included sex, age (years), and ethnicity (White non-Hispanic, Black non-Hispanic, Mexican American, Hispanics/other). Socioeconomic covariates included income (five levels), education (four levels), and marital status (four levels). Lifestyle factors included alcohol consumption (four levels), cigarette smoking (four levels), caffeine intake (mg/day), and dietary intake (methods described in detail below). General health was assessed by body mass index ( $\text{kg}/\text{m}^2$ ), physical activity (PA; created by combining existing variables in the NHANES to assess leisure activity as none, moderate, and vigorous), or use of the following: steroids, bisphosphonates, hormone replacement therapy (HRT, women only), or dietary supplements (any, as well as calcium, vitamin D, or EPA, DHA, SDA individually). Supplement use (y/n) was created as a dichotomous variable to capture use or non-use over the past month of any dietary supplement, calcium, or vitamin D. Supplements included single- and multi-ingredient non-prescription vitamin or mineral supplements, antacids, and prescription supplements. 'Calcium supplement use' (y/n) included supplements explicitly designed to significantly increase total calcium intake, but did not include all supplements containing calcium (i.e., multivitamin and mineral preparation containing calcium). Vitamin D supplement use (y/n) was treated similarly. Omega 3 supplement use (y/n) was limited to EPA, DHA, SDA, or a combination of the three. Therefore, an individual was deemed a n-3 FA supplement user if they were taking any type of fish oil, cod liver oil, marine oil, krill oil, tuna oil, salmon oil, DHA, EPA, or SDA containing supplement over the past 30 days.

All nutrients captured by 24-h recall assessment were evaluated within the regression analyses. Macronutrient intake was measured in grams per day unless otherwise noted and included: energy (kcal/day), carbohydrate, protein, total fat, fiber, cholesterol (mg/day), sugar, saturated fat, monounsaturated fat, and other PUFA (other n-3 and n-6 FA combined). Mineral intake (measured as milligrams per day unless otherwise noted) included: calcium, magnesium, potassium, phosphorous, selenium (mcg/day), zinc, copper, sodium, and iron. Finally, vitamin intake (measured as milligrams per day unless otherwise noted) included: vitamins A (mcg/day), C, E, B12 (mcg/day), and K (mcg/day), total folate (mcg/day), thiamine, pantoic acid, niacin, and riboflavin.

## Statistical analysis

Multivariable regression was applied to investigate the association of dietary n-3 FA with femoral neck BMD, total femur BMD, and lumbar spine BMD. The goal of regression modeling was to identify a combination of covariates in NHANES that explains as much variance in each BMD measure as possible and then to determine whether addition of dietary n-3 FA resulted in a significant increase in variance explained. To accomplish this, a multistage, stepwise approach to model building was used where each BMD site was regressed on the study covariates. The key components of the multistage, stepwise strategy were: (1) classification of potential covariates into related groups (demographics, socioeconomic status, lifestyle factors, general health, macronutrients, minerals, and vitamins); (2) a multistage process where variables were entered into the regression models one group at a time; (3) stepwise removal of covariates that either did not reach statistical significance on entry (using a 5 % threshold) or that were no longer significant with the entry of covariates at later stages; (4) after identification of core variables, stepwise addition of each omitted covariate to determine if it might attain statistical significance or influence statistical significance of other variables in the model; and (5) a final modeling step in which dietary n-3 FA was added to determine whether a significant portion of variance in BMD could be further explained. Interaction terms were tested for sex, age, ethnicity, and BMI in each model.

Regression diagnostics were applied to the final model for each BMD site. All dietary variables were transformed to normality prior to the modeling process. Due to the strong right skew of the dietary omega 3 distribution with many individuals at zero intake, the variable: (dietary omega 3 FA (g/day) +1) was log transformed for its closest transformation to normality. SAS statistical software (version 9.3; SAS Institute, Cary, NC) and STATA statistical software (version 10.0; STATA CORP LP, College Station, TX) were used to perform all statistical analyses.

## Results

### Subject characteristics

Mean ( $\pm$  SEM) values and percentages for subject characteristics at the population level are presented in Table 1. Overall, 2,125 individuals were included in the hip BMD analyses and 1,445 in the spine BMD analyses. Average age was 69.5 years and nearly half of individuals were women and half men. Approximately 70 % of individuals were taking some type of a nutrition supplement. More specifically, approximately 60 % reported taking a calcium supplement, 52 % reported taking a vitamin D supplement, and 15 % reported taking a nutrition supplement containing EPA, DHA, SDA, or combination. Average dietary omega 3 was 0.15 g/day with a range of intake from 0–3.88 g/day. Of the 2,125 individuals with valid hip BMD measurements, approximately 2 % had zero intake of EPA+DHA+SDA, 70 % had intakes between 0.005 and 0.1 g/day, 17 % had intakes between 0.1 and 0.3 g/day, 8.5 % had intakes between 0.3 and 1.0 g/day and only 2 % had omega 3 intakes greater than 1.0 g/day.

### Cross-sectional associations between omega 3 and BMD

Interaction terms between sex and ethnicity; sex and age; and BMI and ethnicity were not significant at any BMD site at the 0.05 significance level. Therefore, these terms were removed from subsequent modeling steps.

The final regression model describing variation in femoral neck BMD ( $R^2=32.4$  %) included the following covariates: age, sex, ethnicity, income, BMI, HRT, PA, alcohol consumption, and dietary magnesium (Table 2). No significant association was observed for n-3 FA supplement use and femoral neck BMD ( $p=0.47$ ). A positive trend between dietary omega 3 (EPA+DHA+SDA) and BMD at the femoral neck was observed, but it was of borderline statistical significance ( $p=0.0505$ ;  $R^2=32.6$  %).

The final model for total femur BMD ( $R^2=44.2$  %) included the following covariates: age, sex, ethnicity, BMI, HTR use, PA, alcohol consumption, smoking status, and dietary magnesium (Table 3). Omega-3 FA supplementation use was not associated with total femur BMD ( $p=0.38$ ) and therefore not included in the final model. In the final model dietary omega 3 was not associated with total femur BMD ( $p=0.95$ ).

The final model for lumbar spine BMD included the following covariates: sex, race, income, BMI, HRT, alcohol consumption, n-3 supplement use, and dietary calcium, with an  $R^2=28.6$  % (Table 4). When added to the model, dietary omega 3 was not statistically significant ( $p=0.74$ ). The lumbar spine was the only BMD site for which n-3 supplement use was statistically significant ( $p=0.005$ ).

## Discussion

Based on a conservative modeling strategy, the 2005–2008 NHANES data provide evidence of a marginally significant, positive association between dietary omega 3 FA (EPA, DHA, and SDA) and BMD of the femoral neck among older adults. In contrast, supplement use of

DHA, EPA, SDA, or their combination, a covariate in this investigation, was overtly significantly associated with better spine BMD. These results suggest that supplemental intake of EPA, DHA, or SDA is associated with greater BMD at the lumbar spine.

Recommendations for EPA and DHA vary by organization. The Institute of Medicine recommends 1.6 g/day of ALA for men and 1.1 g/day for women, of which 10 % should be from EPA and DHA [26]. This is in comparison to the Dietary Guidelines Advisory Committee (496 mg EPA and DHA per day) [27], and the World Health Organization (1–2 % of energy per day from n-3 FA) [28]. Current total dietary omega 3 intake (both marine and non-marine derived) in the USA is estimated at 1.6 g/day where an average of 0.1–0.2 g/day consists of DHA and EPA [4]. These estimations are consistent with the average intake of 0.15 g/day DHA and EPA observed in this study.

Recent epidemiological studies portray a controversial picture of the association between PUFA intake and BMD, where results differ depending upon type of PUFA examined (i.e., total n-3 and n-6, marine-derived omega 3 or other), age of the population and primary outcome (BMD or fracture risk). Farina and colleagues [12] estimated dietary omega 3 and fish intake by food frequency questionnaire and assessed 4 years change in BMD among older adults (mean age 75 years). Overall, high intakes of fish (> 3 servings/week) were associated with maintenance of femoral neck BMD compared to low fish intake. Further, among women with EPA and DHA intakes above or equal to the median, those with the highest AA (n-6) FA intakes had a higher mean baseline femoral neck BMD than those in the lowest quartile. Therefore, the potential protective effects of AA on bone may be dependent upon DHA and EPA intake. In support of these findings, cross-sectional research demonstrated a positive relation between greater seafood consumption (high in n-3 FA) and BMD in pre-menopausal women [29]; a positive relation between serum DHA and BMD in young men [30]; and a positive association with total PUFA intake and BMD among older men and women [31]. Further, this observation was repeated in women specifically not taking HRT [17]; however, no associations between fish specific FA and BMD were observed. In this population omega 3 intake was divided into quartiles where the highest consumption group of EPA and DHA combined was >0.57 g/day. It is important to note that the highest quartile of intake was minimal compared to recommended standards.

Studies examining EPA and DHA intake and risk of fracture are controversial. Orchard et al. [14] observed lower fracture risk with greater total intake of PUFA; however, greater intakes of marine-derived FA were associated with greater total fracture risk. Similarly, reduced hip fracture rates have been observed with higher intakes of AA, but not EPA and DHA [11]. Results from data pooled from the Nurses' Health Study and the Health Professionals Follow-up Study found no significant associations between PUFA intakes and hip fracture risk among men and women combined. However, among women only, lower intakes of total PUFA (7.9 vs 13.9 g/day) were associated with increased risk of hip fracture [16]. No relation with hip fracture was observed when fish intake and EPA and DHA were examined separately [15].

The current study found a positive association between the use of a supplement containing DHA, EPA, or their combination and greater BMD at the spine. A typical fish oil supplement contains approximately 0.3 g DHA and EPA [32], which is approximately double the level of DHA and EPA taken from dietary sources in this study. Few clinical intervention trials in humans supplementing PUFA support this association. Terano et al. [33] found women receiving 1.8 g of EPA supplement for 12 months had a significant increase in the transition index (a measure of bone density) from a quantitative heel ultrasound. However, Basse and colleagues [34] found a combination supplement of n-3 and n-6 FA for 12 months did not alter BMD among pre- and post-menopausal women.

More recently, Appleton et al. [35] randomized men and women <50 years to 1.48 g EPA and DHA or placebo for 12 weeks. C-terminal cross-linking telopeptide of type 1 collagen (bone turnover maker) was not associated with omega 3 intake, although the lack of effect may be due to the young study sample. Change in bone metabolism may be better captured in postmenopausal women where bone is more metabolically active and the protective effects of estrogen are lost [8].

There are several plausible mechanisms describing how n-3 FA may be protective of bone health. The activation of nuclear factor kappa B (NF- $\kappa$ B) mediates an overall increase in bone resorption and decrease in formation, therefore creating a global reduction in bone mass. Recent research utilizing fat-1 transgenic mice have shown n-3 FA to be protective against NF- $\kappa$ B translocation and activation [36, 37]. The ability of long-chain PUFA (specifically EPA and DHA) to reduce NF- $\kappa$ B activity has been shown to decrease osteoclast maturation and therefore, slow bone resorption. Animal research in chicks [38] and rats [39] found decreasing the dietary n6:n3 ratio decreases *ex vivo* production of prostaglandin E2 (PGE2, a pro-inflammatory prostaglandin) and increases markers of bone formation, suggesting n-3 FA may be protective of bone by inhibiting detrimental cytokine production. It is also speculated that the protective effect of EPA on osteoblast maturation may be mediated by cross-talk between PGE2 and nitric oxide production [7]. PUFA may also positively influence bone metabolism by inhibiting the action of other transcription factors such as peroxisome proliferator activator receptor  $\gamma$  (PPAR $\gamma$ ). This transcription factor favors the maturation of pre-osteoblasts into adipocytes, therefore reducing osteoblast formation. Long-chain PUFA, specifically DHA, have been shown to augment osteoblast maturation by reducing PPAR $\gamma$  activity [40].

The current study has inherent strengths and limitations. Data from NHANES are generalizable to both men and women of multiple ethnic groups. Due to the cross-sectional nature of this study, it is unclear whether the statistically equivocal evidence of a positive association between high intakes of dietary omega 3 and femoral neck BMD and the more definitive evidence of an association between omega 3 supplement use and higher spine BMD signal causal relations or reflect confounding due to healthier lifestyles led by those with high intakes of omega 3. In this study, a conservative statistical methodology was employed to account for variance in BMD by covariates that may capture healthy lifestyle factors and to reduce the possibility of false positive findings for the omega 3-BMD association via confounding. We do not have information on how long n-3 FA supplements were taken. Further, due to the complexity of omega 3 supplements with blended ingredients we were unable to calculate the dosage of EPA, DHA, and SDA taken by individuals in this sample. Therefore, these factors could lead to residual confounding.

Dietary intake was assessed by two, 24-h recall assessments and averaged over the 2 days. Within-person variation is not fully accounted for during the averaging process and may create excess noise in the estimates of dietary intake. Methods assessing usual intake of nutrients by accounting for such variation are available [41, 42], and rely on the Box-Cox transformation to determine the distribution of usual intake for a nutrient. However, these methods of assessment, to our knowledge, provide no remedy for a situation where the Box-Cox transformation cannot successfully transform observed “non-zero” intake values to approximate normality (as was the case for the present study). The within-person variation that is apt to persist in the 2-day average method used in this paper is likely to bias statistical testing results for a nutrient toward the null hypothesis. Lastly, low vitamin D is a known risk factor for low bone mass; however, we were unable to account for either serum vitamin D or dietary vitamin D because this information has yet to be released by the NHANES for all years used in the current analysis.

Overall, the results of this study suggest that intakes of omega 3 FA may be associated with higher BMD at the femoral neck. Additionally, use of omega 3 supplements containing EPA, DHA, SDA, or their combination has a positive impact on BMD at the spine in older adults. Further assessment of supplement intake (amount taken per day) would be helpful to determine whether levels of marine-derived omega 3 at supplemental doses are associated with greater BMD at the hip.

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**Table 1**Subject characteristics, mean  $\pm$  SEM or percent,  $n = 2,125$ 

<b>Descriptive variables</b>	<b>Population estimate<sup>a</sup></b>
Demographics	
Age (years)	69.5 $\pm$ 0.20
Gender (%)	
Women	48.3 $\pm$ 1.1
Men	51.7 $\pm$ 1.1
Ethnicity (%)	
Mexican American	4.4 $\pm$ 0.6
Hispanic and other	5.7 $\pm$ 1.1
White non-Hispanic	81.3 $\pm$ 2.1
Black non-Hispanic	8.6 $\pm$ 1.2
Socioeconomic status	
Income (%)	
<\$25,000	16 $\pm$ 1
<\$45,000	37 $\pm$ 2
<\$75,000	19 $\pm$ 1
\$75,000	21 $\pm$ 2
Refused/don't know	7 $\pm$ 1
Lifestyle factors	
Alcohol consumption (%)	
<1 drink/month or unknown	61 $\pm$ 3
1 drink/month and <1 drink/week	9 $\pm$ 1
1 drink/week and <1 drink/day	23 $\pm$ 2
1 drink per day	7 $\pm$ 1
Smoking status (%)	
Never	44 $\pm$ 1
Former	43 $\pm$ 2
Current, 1 pack per day	11 $\pm$ 1
Current, >1 pack per day	2 $\pm$ 1
Caffeine (mg/day)	166 $\pm$ 5
General health	
Body mass index (kg/m <sup>2</sup> )	28.1 $\pm$ 0.16
Dietary supplement use (%)	
Yes	70 $\pm$ 2
No	30 $\pm$ 2
Calcium supplement use (%)	
Yes	61 $\pm$ 2
No	39 $\pm$ 2
Vitamin D supplement use (%)	
Yes	52 $\pm$ 2

<b>Descriptive variables</b>	<b>Population estimate<sup>a</sup></b>
No	48±2
Omega 3 supplement use (%)	
Yes	15±1
No	85±1
HRT use (%)	
Yes	4±1
No	96±1
Physical activity leisure (%)	
None	48±2
Moderate	37±1
Vigorous	15±2
Nutrient intake	
Energy (kcal/day)	1,814±29.8
Omega 3 (g/day)	0.15±0.02
Calcium (mg/day)	846±18.4
Magnesium (mg/day)	279±5.7
Bone mineral density (g/cm <sup>2</sup> )	
Total femur	0.76±0.004
Femoral neck	0.92±0.005
Total spine	1.03±0.007

*HRT* hormone replacement therapy in the population

<sup>a</sup>Percentages are reported ± their SE because they are descriptive statistics that are sample-based estimates for the US population

**Table 2**

Final model parameters from multistage, stepwise regression analyses assessing the relation between dietary omega 3 and femoral neck bone mineral density (BMD),  $n = 2,125$

	Femoral neck BMD <sup>a</sup> $\beta \pm SE$	<i>p</i> value
Model $R^2$	32.6 %	
Age	-0.0012 $\pm$ 0.0003	<0.001 *
Gender	-0.0399 $\pm$ 0.0038	<0.001 *
Ethnicity		<0.001 *
Mexican (ref)	-	
Hispanic and other	-0.0060 $\pm$ 0.0077	
White	-0.0099 $\pm$ 0.0044	
Black	0.0385 $\pm$ 0.0053	
Income		0.005*
Level 1 (ref)	-	
Level 2	0.0152 $\pm$ 0.0053	
Level 3	0.0086 $\pm$ 0.0053	
Level 4	0.0153 $\pm$ 0.0068	
Level 5	0.0259 $\pm$ 0.0063	
BMI	0.0059 $\pm$ 0.0003	<0.001 *
HRT	0.0511 $\pm$ 0.0093	<0.001 *
Physical activity		0.047*
None (ref)	-	
Moderate	0.0036 $\pm$ 0.0037	
Vigorous	0.0138 $\pm$ 0.0053	
Alcohol consumption		0.007*
<1 drink/month or unknown (ref)	-	
1 drink/month and <1 drink/week	0.0134 $\pm$ 0.0036	
1 drink/week and <1 drink/day	0.0126 $\pm$ 0.0057	
1 drink per day	0.0099 $\pm$ 0.0058	
Dietary magnesium <sup>a</sup>	0.0048 $\pm$ 0.0025	0.064
Marine-derived dietary omega 3 <sup>a</sup>	0.0397 $\pm$ 0.0195	0.0505

HRT hormone replacement therapy use, BMI body mass index

\*  $p < 0.05$

<sup>a</sup>Femoral neck BMD log transformed; dietary magnesium cube root transformed, marine derived dietary omega 3 presented as the log of reported intakes plus 1 to correct for distribution skewness

**Table 3**

Final model parameters from multistage, stepwise regression analyses assessing the relation between dietary omega 3 and total femur bone mineral density (BMD),  $n = 2,125$

	Total femur BMD <sup>a</sup> $\beta \pm SE$	<i>p</i> value
Model <i>R</i> <sup>2</sup>	44.2 %	
Age	-0.0013±0.0003	<0.001 *
Gender	-0.0705±0.0040	<0.001 *
Ethnicity		<0.001 *
Mexican (ref)	–	
Hispanic and other	0.0065±0.0076	
White	-0.0006±0.0047	
Black	0.0355±0.0049	
BMI	0.0063±0.0003	<0.001 *
HRT	0.0573±0.0075	<0.001 *
Physical activity		0.006 *
None (ref)	–	
Moderate	0.0075±0.0033	
Vigorous	0.0198±0.0058	
Alcohol consumption		<0.001 *
<1 drink/month or unknown (ref)	–	
1 drink/month and <1 drink/week	0.0156±0.0036	
1 drink/week and <1 drink/day	0.0166±0.0050	
1 drink per day	0.0125±0.0065	
Smoking status		0.022 *
Never (ref)	–	
Former	-0.0042±0.0034	
1 pack per day	-0.0171±0.0072	
>1 pack per day	-0.0309±0.0105	
Dietary magnesium <sup>a</sup>	0.0074±0.0030	0.018 *
Marine derived dietary omega 3 <sup>a</sup>	-0.0011±0.0179	0.950

*HRT* hormone replacement therapy use, *BMI* body mass index

\* *p* < 0.05

<sup>a</sup>Total femur BMD square root transformed; dietary magnesium cube root transformed, marine-derived dietary omega 3 presented as the log of reported intakes plus 1 to correct for distribution skewness

**Table 4**

Final model parameters from multistage, stepwise regression analyses assessing the relation between dietary omega 3 and lumbar spine bone mineral density (BMD),  $n = 1,445$

	Lumbar spine BMD <sup>a</sup> $\beta \pm SE$	<i>p</i> value
Model $R^2$	28.6 %	
Gender	-0.0587±0.0056	<0.001*
Ethnicity		<0.001*
Mexican (ref)	–	
Hispanic and other	0.0142±0.0114	
White	0.0290±0.0073	
Black	0.0726±0.0090	
Income		0.044*
Level 1 (ref)	–	
Level 2	0.0229±0.0067	
Level 3	0.0206±0.0080	
Level 4	0.0169±0.0079	
Level 5	0.0206±0.0106	
BMI	0.0050±0.0004	<0.001*
HRT	0.0707±0.0163	<0.001*
Alcohol consumption		<0.005*
<1 drink/month or unknown (ref)	–	
1 drink/month and <1 drink/week	0.0413±0.0118	
1 drink/week and <1 drink/day	0.0148±0.0054	
1 drink per day	0.0233±0.0106	
Dietary calcium <sup>a</sup>	0.0079±0.0039	0.051
Omega 3 supplement use	0.0211±0.0276	0.005*
Marine derived dietary omega 3 <sup>a</sup>	0.0091±0.0276	0.743

HRT hormone replacement therapy use, BMI body mass index

\*  $p < 0.05$

<sup>a</sup>Lumbar spine BMD square root transformed; dietary calcium fourth root transformed, marine-derived dietary omega 3 presented as the log of reported intakes plus 1 to correct for distribution skewness