European Journal of Nutrition
https://doi.org/10.1007/s00394-018-1665-6

ORIGINAL CONTRIBUTION

Total and lean fish intake is positively associated with bone mineral density in older women in the community-based Hordaland Health Study

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Received: 1 November 2017 / Accepted: 9 March 2018

Abstract

Purpose Fish is a source of various nutrients beneficial for bone health, but few studies have investigated the association between bone mineral density (BMD) and fish consumption. Thus, the aim was to investigate the relationship between total fish intake and BMD and between both lean and fatty fish intake and BMD.

Method These cross-sectional analyses include 4656 participants in the Hordaland Health Study, a community-based study conducted in 1997–1999. The study includes two birth cohorts of men and women from Hordaland county (Norway) born in 1950–1951 and 1925–1927. BMD was measured by dual-energy X-ray absorptiometry and dietary intake was obtained from a semi-quantitative food-frequency questionnaire.

Results The average total fish intake was 33 ± 18 g/1000 kcal and was primarily lean fish. Older women had significantly lower BMD than older men and middle-aged men and women. In older women, total and lean fish intake (50 g/1000 kcal) was significantly and positively associated with BMD also after multivariate adjustments (β-coefficient 0.018, $p = 0.017$ and 0.026, $p = 0.021$).

Conclusion A high intake of fish, in particular lean fish, was positively associated with BMD in older women. No association between intake of fatty fish and BMD was found in either of the age and sex groups.

Keywords Diet · Food-frequency questionnaire · Fatty fish · Lean fish · Bone mineral density · Osteoporosis

Abbreviations

BMD	Bone mineral density
DHA	Docosahexaenoic acid
DPA	Docosapentaenoic acid
DXA	Dual-energy X-ray absorptiometry
EPA	Eicosapentaenoic acid
FFQ	Food-frequency questionnaire
HUSK	Hordaland Health Study
NNR 2012	Nordic Nutritional Recommendation 2012
n3 PUFA	Omega-3 polyunsaturated fatty acids

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Introduction

Osteoporosis is a major public health challenge, especially in an aging population, with the most severe consequence being fractures of the hip, wrist, or spine. The diagnosis of osteoporosis is made either after a low-energy fracture or by measurement of bone mineral density (BMD), preferably by dual-energy X-ray absorptiometry (DXA) technique. A BMD below 2.5 standard deviations of the average of young healthy adults is indicative for osteoporosis, applying age, and sex-specific cutoffs [1, 2]. In humans, BMD reaches

Published online: 15 March 2018

its peak in the third decade of life [3], and it decreases throughout life, with the fastest decline among women in the peri- and early postmenopausal state. This could be due to loss of endogenous estrogen, which in turn is associated with increased production of pro-inflammatory cytokines as mediators for the accelerated bone loss. It could also be due to other cell-autonomous age-related factors [4]. Men have higher BMD levels than women of similar age due to larger bones and thicker bone cortex [5].

Non-modifiable risk factors for low BMD include old age, female gender, and genetics, where genetics is proposed to predict 60–80% of the variability in bone mass [6]. Genetic risk factors include family history of osteoporosis [7, 8], ethnic differences in BMD [9, 10], and individual genetic variations [11]. Modifiable factors associated with low BMD are low lean body mass, alcohol consumption, smoking, physical inactivity, and use of osteoporosis inducing drugs like glucocorticoids. In recent years, diet has been given attention as a modifiable risk factor associated with bone health throughout life [6]. Although the role of specific nutrients or foods is debated, there is reasonable consensus that calcium, vitamin D [12, 13], vitamin K [14, 15], and perhaps n3 PUFA [16, 17] are important for bone health. Recently, the National Osteoporosis Foundation stated that there is moderate-to-strong evidence for calcium, vitamin D, and dairy consumption having a positive effect on peak bone mass [6]. The role of protein intake is a matter of debate, but recently, a high protein intake was reported to be associated with higher bone mass [18, 19]. Fish is a good source of nutrients associated with prevention of osteoporosis, such as high-quality protein, n3 PUFA, and vitamin D. Only a few studies have investigated the association between fish consumption and BMD. In the Framingham Osteoporosis Study, a protective effect of high intake of fish (≥ 3 servings/week) was found on bone loss [20]. In another large US prospective cohort of older adults, high fish consumption was associated with lower BMD [21]. Two Chinese studies found beneficial effects of fish intake on BMD and risk of osteoporosis [22, 23]. In addition, in Spanish premenopausal women, a positive association between fish intake and BMD was reported [24]. Due to these conflicting data, there is a need for more studies on the effect of fish intake in different populations with marked variations in both BMD and fish intake. Fish is a heterogeneous food group and populations with high total fish intake allow analysis of different types of fish. In general, Norwegians have a high intake of fish, with higher intake in older than in younger groups [25, 26]. Due to the habitually high fish intake in Norway, the present cohort is well suited to explore the association between BMD and overall fish intake, as well as lean and fatty fish intake. Thus, the main objective of this cross-sectional study was to investigate the relationship between intake of total, lean, and fatty fish and BMD in the Hordaland Health Study (HUSK).

Subjects and methods

Study population

The current work is a cross-sectional study of the large community-based Norwegian Hordaland Health Study (HUSK). HUSK was conducted from 1997 to 1999 as a collaboration between the University of Bergen, University of Oslo, local health services, and the Norwegian Institute of Public Health. Participants in HUSK are from two birth cohorts born either in 1925–1927 (older cohort) or in 1950–1951 (middle-aged cohort) from Hordaland county in Western Norway.

In 1997–1999, information on dietary intake and bone mineral density (BMD) was collected in about 4700 participants, allowing analysis of the association between dietary intake and BMD in both middle-aged and older men and women. More information about HUSK can be found at http://husk-en.b.uib.no/.

Dietary assessment

Habitual dietary intake (reflecting the previous year) was estimated using a 169-item FFQ developed at the Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo [27]. The questionnaire was handed out on the day of the health examination and then filled out at home. The questionnaire was later mailed to the HUSK Project Center in Bergen. Portion size was considered (e.g. slice, glass etc.) and questions on supplement use were included in the FFQ. Daily food (including fish consumption) and nutrient intakes were calculated using a food database and software system (Kostberegningssystem, version 3.2; University of Oslo, Norway). The FFQ has been compared against a weighted dietary record and fatty acid composition in serum phospholipids [28, 29]. For the dietary intake, an energy intake lower than 700 or 800 kcal and higher than 360 or 4200 kcal for women and men, respectively, was considered unreasonable and removed from the analysis, leaving 4656 participants with dietary records.

The questions related to dietary fish intake have been described in detail elsewhere [30]. Briefly, in addition to total fish (without shellfish), fish intake was divided into fatty fish (herring, mackerel, salmon, trout, and fish used as spread) and lean fish (cod, pollock, and haddock). The nutrient density method was used for energy adjustments [31] of all dietary variables and either stated as g/1000 kcal or percentage of total energy intake. In the multiple linear regression models, total fish, lean fish, and fatty fish were presented as 50 g/1000 kcal. The total

marine n3 PUFA intake was calculated by combining the variables for eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA).

Use of fish oil, cod liver oil (oil or capsules), calcium supplements, and vitamin D supplements was assessed in the FFQ. Participants who reported using such supplements more than once a week were defined as users. Alcohol intake was self-reported and was converted into g/day. Sex-specific cutoffs were used, and one unit of alcohol was defined as 10 g/day in accordance with the Nordic Nutrition Recommendations 2012 [32]. The intake was grouped into four categories; 0 = 0 g/day; 1 = women: > 0–10 g/day; men: > 0–20 g/day; 2 = women: > 10–20 g/day; men: > 20–30 g/day; 3 = women: > 20 g/day; men: > 30 g/day.

Clinical data

The HUSK measurements included a measurement of BMD of 5377 participants by DXA. The DXA measurement was performed at a different appointment after the initial visit to the Project Center. Measurements of BMD have been described in detail elsewhere [33]. Briefly, BMD of the femoral neck and total hip (g/cm^2) was measured by a DXA (EXPERT-XL; Lunar Company Inc, Madison, Wis, USA). The left hip was scanned unless there was a history of the previous fracture or surgery. The DXA measurements also allow calculation of body composition, that is fat mass and lean mass [34]. Weight and height were measured with the participants wearing light clothing without shoes, to the nearest 0.5 kg and 1 cm, respectively.

Covariate assessment

Self-administered questionnaires provided information regarding current estrogen therapy, physical activity (hard and light), and smoking (current/former/never smoked). Physical activity was categorized as by Vinknes et al. [35]. Categories for light physical activity were 0 (none), 0.25 (< 1 h/week), 0.5 (1–2 h/week), or 1.0 (≥ 3 h/week) and for hard physical activity were 0 (none), 0.5 (< 1 h/week), 1.0 (1–2 h/week), or 2.0 (≥ 3 h/week). The sum of these scores was calculated and used in the multivariate models. Smoking habits were categorized as current smoker, former smoker, and never smoker. In addition to the self-reported smoking habits, cotinine was measured as a marker of recent nicotine exposure. Cotinine was measured in EDTA plasma stored at − 80 °C until analyzed at Bevital A/S (http://www.bevital.no), Bergen, Norway by LC/MS/MS. Smokers were defined due to cotinine levels ≥ 85 nmol/L [36, 37].

Statistical analysis

Continuous variables are presented as means and standard deviation and categorical variables as percentages. Differences between sex and age were assessed using Mann–Whitney *U* test for continuous variables and Fischer's exact test for categorical variables. Fish intake was also categorized into quartiles, calculated separately for each age group and sex. Differences in characteristics across quartiles of total fish intake were analyzed using linear regression for continuous variables and logistic regression for dichotomous variables.

The association of fish intake with BMD was analyzed both in quartiles of fish intake and fish intake as continuous variable. Multiple linear regression analyses were performed to assess the association between intake of either total, fatty, or lean fish and BMD (by age group and sex) with adjustment for potential confounders. Due to missing values in confounders (1.3–6.2%), the multivariate analysis included 4279 participants, whereas the energy-adjusted model included 4656 participants. The number of nonconsumers of lean and fatty fish was low, 405 (8.7%) and 255 (5.5%), respectively. The nonconsumers were categorized in quartile one.

The statistical software SPSS for Windows version 22 (IBM, NY, USA) was used for statistical analyses. A two-sided p value < 0.05 was considered statistically significant.

Results

The current analysis is based on 4656 participants from the HUSK study. Eligibility and selection of participants are shown in Fig. 1.

Characteristics

Characteristics of the study population, stratified by age group and sex, are presented in Table 1. There were more women (57%) than men in the total cohort. Femoral neck BMD and total hip BMD were higher in men than in women and higher in the middle-age cohorts than in the older.

A significantly higher proportion of both middle-aged and older women did not engage in hard physical activity in their leisure time, compared to men in the same age group. In the older individuals, men were more likely to be former smokers than women, while a higher proportion of women had never smoked. Significantly, more women than men reported no alcohol consumption. Men had lower fat mass and higher lean mass than women, and the older women had significantly more fat mass and lower lean mass than the

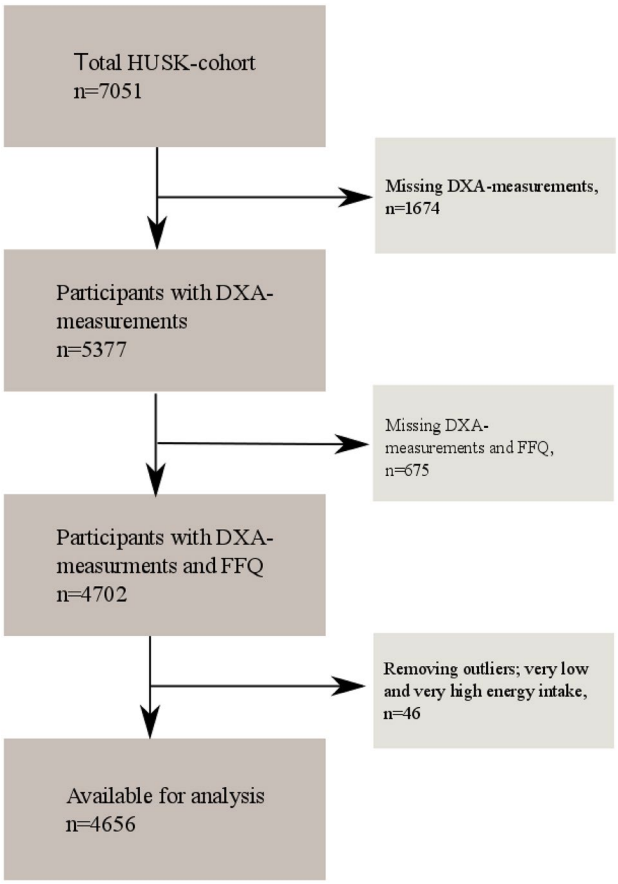

Fig. 1 Flowchart of the study population available for analysis from the Hordaland Health Study (HUSK). *DXA* dual-energy x-ray absorptiometry; *FFQ* food frequency questionnaire

middle-aged women. About one in six women used estrogen therapy.

Dietary intake by quartiles of fish intake

Fish intake was categorized into sex- and age-specific quartiles. Dietary intake for nutrients and food groups are presented across quartiles of fish intake in Table 2 (middle-aged and older men) and Table 3 (middle-aged and older women). Energy intake was not different between fish intake across the quartiles in any age or sex group. Older men had the highest total fish intake of all the age and sex groups. The intake of lean fish in older men was almost twice the intake in middle-aged men. There were also small, but significant differences in fatty fish intake. Age group differences in the female cohorts were less pronounced, but the older women had higher total fish intake and higher lean fish intake than the middle- aged women.

Dairy intake was similar in all the quartiles of fish intake in men, but among women, high fish consumers had low consumption of milk products. For all groups, high fish consumers usually had a higher meat intake than low fish consumers. High fish consumers also had a considerably higher vegetable intake than the low fish consumers. There was no difference in fruit and berries consumption across the quartiles among men or women nor between the two age groups.

In the total cohort, 36% used cod liver oil weekly, with the older men having the highest intake (40%). Supplementation of fish oil and vitamin D was not common. In the older women, 13% used calcium supplements, which is considerably higher than in the other groups.

Intake of vitamin D and n3 PUFA from food and supplements, and protein intake (both as energy percent and as g per kg body weight) increased across quartiles of fish intake in all cohorts. There was no difference in total calcium intake across quartiles.

Fish intake and bone mineral density

The Spearman's rho between total hip BMD and femoral neck BMD was in middle-aged men: $r = 0.90$, older men: $r = 0.89$, middle-aged women: $r = 0.88$ and older women: $r = 0.86$. Use of total hip BMD as outcome variable did not change the results substantially (data not shown). Femoral neck BMD increased across quartiles of fish intake in middle-aged men and older women, while there was no significant association in middle-aged women or older men (Fig. 2).

The linear regression analysis on the association of fish intake with femoral neck BMD is presented in Table 4. In middle-aged men and older women, total fish intake was positively associated with BMD (Model 1; adjusted for total energy intake). After additional adjustments for BMI, physical activity score, cotinine > 85 nmol/L, and alcohol consumption (Model 2), the association was no longer significant for middle-aged men, but remained significant in the older women. Further analysis of the type of fish revealed that high lean fish intake was significantly associated with high femoral neck BMD in older women in the fully adjusted model, whereas fatty fish did not show such an association. A sensitivity analysis leaving out the nonconsumers, did not change the results (data not shown).

Discussion

In this population-based study in Norwegians with high habitual fish intake, a positive significant association between total fish intake and BMD was observed in older women (70–74 years), but not in older men or in the middle-aged cohorts (46–49 years). The effect remained stable even after adjustment for various covariates known to be associated with BMD. The association of total fish intake with

Table 1 Characteristics of 4656 middle-aged (46–49 years) and older (70–74 years) men and women in the Hordaland Health Study

	Total cohort	Middle-aged		Older	
		Men ($n=1052$)	Women ($n=1605$)	Men ($n=962$)	Women ($n=1037$)
Age (years)		47 ± 1	47 ± 1	72 ± 1	72 ± 1
Women (%)	56.7				
Weight (kg)[a,c,d,e]	74.1 ± 13.4	84.0 ± 11.9	68.3 ± 1.6	79.7 ± 11.1	67.6 ± 11.2
Height (m)[a,c,d,e,f]	1.70 ± 0.09	1.79 ± 0.06	1.66 ± 0.06	1.75 ± 0.06	1.61 ± 0.05
BMI (kg/m^2)[a,,c,f]	25.7 ± 3.8	26.2 ± 3.3	24.8 ± 4.0	26.0 ± 3.2	26.2 ± 4.2
Fat mass (kg)[a,c,d,e,f]	23.4 ± 9.6	20.5 ± 9.1	24.4 ± 9.6	21.3 ± 8.6	26.9 ± 9.5
Lean mass (kg)[a,c,d,e,f]	47.2 ± 10.6	59.9 ± 6.2	40.3 ± 4.5	55.1 ± 5.8	37.7 ± 4.3
Femoral neck BMD (g/cm^2)[c,d,e,f]	0.911 ± 0.15	1.000 ± 0.14	0.961 ± 0.12	0.901 ± 0.12	0.763 ± 0.11
Total hip BMD (g/cm^2)[c,d,e,f]	0.950 ± 0.16	1.031 ± 0.14	0.986 ± 0.13	0.962 ± 0.15	0.800 ± 0.12
Hard physical activity (%)[a]					
None [c,d,e,f]	3.5	2.4	4.2	2.4	5.6
<1 h/week[c,d,e,f]	22.2	20.4	18.5	19.5	32.7
1–2 h/week[d,e]	45.9	45.2	45.1	47.6	44.5
≥3 h/week[c,d,f]	28.4	32.0	32.2	30.4	17.2
Smoking habits (%)					
Current smoker[d,e,f]	27.4	35.4	36.3	17.8	14.5
Former smoker[c,d,e]	35.2	33.4	26.6	60.7	26.8
Never smoked[c,d,e,f]	40.0	35.6	40.0	24.6	60.4
Cotinine ≥ 85 nmol/L (%)[a,e,f]	27.4	35.3	36.4	17.7	14.5
Alcohol categories (%)[b]					
None [c,d,e,f]	27.5	9.1	20.6	29.8	53.5
Low [c,d,e,f]	63.5	80.0	67.7	62.2	41.5
Moderate[c,e,f]	6.7	6.7	10.0	4.0	4.3
High[s,d,f]	2.7	4.2	2.3	4.1	0.7
Current estrogen therapy (for women) (%)[f]	16.8	NA	17.9	NA	15.1

Values represented mean ± SD or %. *p* Values for the difference between the age and sex groups were calculated using Mann–Whitney *U* test or Fischer exact test

[a] $n=4279$–4653

[b] None: 0 g/day, low: women: > 0–10 g, men: > 0–20 g, moderate: women: > 10–20 g, men: > 20–30 g, high: women: > 20 g, men: > 30 g

[c] Significant ($p \leq 0.05$) difference between the middle-age by sex

[d] Significant ($p \leq 0.05$) difference between the older by sex

[e] Significant ($p \leq 0.05$) difference between the men by age

[f] Significant ($p \leq 0.05$) difference between the women by age

BMD was mainly due to the high intake of lean fish, whereas fatty fish intake was not significantly associated with BMD in any of the cohorts.

Comparison with other studies

Although several studies have focused on fish intake and BMD, these studies are difficult to compare due to differences in age range, sex, and ethnicity. There are also a variety in amount and type of fish consumed, and how fish intake is monitored. In the present study, the association of fish intake with BMD was evaluated from dietary intake data obtained from an FFQ, energy-adjusted and analyzed as a single food group, but with separate analyses for fatty and lean fish. A similar approach has been used in predominantly Caucasian populations from the US and Europe [20, 21, 24, 38] and in Asian cohorts [22, 23, 38, 39]. With the exception of the study by Virtanen et al. [21], these studies report a positive association of fish intake and BMD, despite differences in fish classification and assessment of dietary fish intake. However, the association was only significant in the postmenopausal Chinese women with very high fish intake [22], in old rural Chinese women with fish intake > 250 g/week [23], in the high consuming Spanish premenopausal women [24], and in Koreans reporting high fish intake [38]. The latter study showed that many other factors were important for BMD in old adults, as despite high fish intake and positive associations with BMD in the Koreans, the absolute

Table 2 Dietary characteristics and measures of BMD in 1052 middle-aged (46–49 years) and 962 older (70–74 years) men by quartiles of total fish intake in the Hordaland Health Study

Total fish, g (min–max)	Middle-aged men					*p* for trend	Older men					*p* for trend
	Total	1st quartile (*n* = 263) 1.1–20.9	2nd quartile (*n* = 263) 20.9–30.6	3rd quartile (*n* = 263) 30.6–42.5	4th quartile (*n* = 263) 42.5–162.7		Total	1st quartile (*n* = 240) 3.0–33.0	2nd quartile (*n* = 241) 33.1–46.7	3rd quartile (*n* = 241) 46.7–64.1	4th quartile (*n* = 240) 64.3–186.3	
Total energy (kcal)	2533 ± 697	2487 ± 752	2621 ± 654	2527 ± 608	2496 ± 759	0.731	2082 ± 573	2062 ± 559	2125 ± 602	2140 ± 579	1999 ± 543	0.291
Protein (E%)	15.7 ± 2.2	14.5 ± 2.1	15.2 ± 1.7	16.0 ± 1.8	17.3 ± 2.0	< 0.001	16.1 ± 2.3	14.4 ± 1.9	15.6 ± 1.8	16.3 ± 1.7	18.0 ± 2.1	< 0.001
n-3 long chained PUFA (E%)[a]	0.07 ± 0.07	0.04 ± 0.06	0.05 ± 0.04	0.07 ± 0.06	0.1 ± 0.09	< 0.001	0.11 ± 0.10	0.06 ± 0.06	0.09 ± 0.09	0.11 ± 0.08	0.16 ± 0.11	< 0.001
Protein g/kg body weight	1.2 ± 0.4	1.1 ± 0.4	1.2 ± 0.3	1.2 ± 0.3	1.3 ± 0.4	< 0.001	1.1 ± 0.3	1.0 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	1.1 ± 0.4	< 0.001
Vitamin D (g/1000 kcal)[b]	4 ± 3	4 ± 3	4 ± 3	4 ± 3	6 ± 4	< 0.001	6 ± 4	4 ± 3	5 ± 4	6 ± 4	7 ± 5	< 0.001
Calcium (g/1000 kcal)[b]	384 ± 120	392 ± 133	378 ± 119	386 ± 111	378 ± 115	0.552	377 ± 119	451 ± 136	390 ± 137	385 ± 123	369 ± 101	0.004
Supplements (%)												
Fish oil	8.4	7.2	4.9	9.1	12.2	0.026	5.3	3.8	5.4	4.6	7.5	0.304
Cod liver oil	38.9	35.7	38.9	38.4	42.6	0.452	40.1	30.8	43.2	46.5	40.0	0.004
Vitamin D	1.5	0	2.7	1.5	1.9	0.831	1.9	0.8	3.3	0.8	2.5	0.148
Calcium	1.4	0.8	1.9	1.1	1.9	0.630	2.8	2.1	2.5	2.9	3.8	0.725
Food intake (g/1000 kcal)												
Total fish	33 ± 18	13 ± 5	26 ± 3	36 ± 3	58 ± 16	< 0.001	51 ± 25	23 ± 7	40 ± 4	55 ± 5	85 ± 20	< 0.001
Lean fish	12 ± 10	4 ± 4	9 ± 5	14 ± 7	21 ± 13	< 0.001	23 ± 17	9 ± 7	17 ± 8	24 ± 10	40 ± 19	< 0.001
Fatty fish	10 ± 10	2 ± 2	4 ± 4	6 ± 5	11 ± 9	< 0.001	15 ± 13	3 ± 4	6 ± 6	9 ± 7	16 ± 13	< 0.001
Vegetables	79 ± 61	66 ± 58	73 ± 59	83 ± 57	95 ± 66	< 0.001	91 ± 59	79 ± 54	88 ± 60	91 ± 50	106 ± 69	< 0.001
Fruit and berries	97 ± 65	94 ± 68	95 ± 59	95 ± 63	105 ± 68	0.063	116 ± 72	112 ± 73	117 ± 71	115 ± 67	118 ± 78	0.408
Meat	58 ± 23	56 ± 26	57 ± 21	59 ± 21	60 ± 23	0.029	45 ± 21	41 ± 21	46 ± 21	49 ± 20	46 ± 22	0.004
Milk products	156 ± 106	158 ± 118	157 ± 104	156 ± 101	155 ± 103	0.714	162 ± 105	167 ± 114	172 ± 107	159 ± 95	149 ± 104	0.031
Egg	7 ± 5	7 ± 6	7 ± 4	7 ± 5	8 ± 5	0.068	9 ± 7	8 ± 7	10 ± 8	8 ± 5	9 ± 6	0.793

The values represented mean ± SD or %. P for trend: linear regression for continuous variables and logistic regression for dichotomous variables

[a]Sum of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)

[b]Total intake from diet and supplements

Table 3 Dietary characteristics of 1605 middle-aged (46–49 years) and 1037 older (70–73 years) women by quartiles of total fish intake in the Hordaland Health Study

Total fish, g (min–max)	Middle-aged women					*p* for trend	Older women					*p* for trend
	Total	1st quartile (*n* = 401) 0.8–22.3	2nd quartile (*n* = 401) 22.3–32.3	3rd quartile (*n* = 402) 32.3–44.9	4th quartile (*n* = 401) 45.0–144.1		Total	1st quartile (*n* = 259) 0.9–27.6	2nd quartile (*n* = 259) 27.7–40.8	3rd quartile (*n* = 260) 40.9–56.5	4th quartile (*n* = 259) 56.9–189.6	
Total energy (kcal)	1914 ± 566	1894 ± 552	1955 ± 542	1891 ± 482	1918 ± 672	0.957	1627 ± 497	1572 ± 505	1628 ± 483	1673 ± 487	1636 ± 510	0.085
Protein (E%)	16.4 ± 2.4	15.1 ± 2.2	15.7 ± 1.9	16.6 ± 1.9	18.1 ± 2.3	< 0.001	16.5 ± 2.6	14.8 ± 2.2	15.9 ± 2.0	16.8 ± 2.0	18.4 ± 2.5	< 0.001
Protein g/kg body weight	1.2 ± 0.4	1.1 ± 0.4	1.1 ± 0.4	1.1 ± 0.3	1.3 ± 0.5	< 0.001	1.0 ± 0.4	0.9 ± 0.4	1.0 ± 0.3	1.0 ± 0.3	1.1 ± 0.4	< 0.001
n-3 long chained PUFA (E%)[a]	0.09 ± 0.10	0.06 ± 0.08	0.07 ± 0.08	0.09 ± 0.10	0.12 ± 0.13	< 0.001	0.09 ± 0.12	0.10 ± 0.10	0.11 ± 0.10	0.11 ± 0.10	0.17 ± 0.13	< 0.001
Vitamin D (g/1000 kcal)[b]	5 ± 4	4 ± 4	4 ± 4	5 ± 4	6 ± 4	< 0.001	5 ± 4	4 ± 5	5 ± 4	5 ± 4	7 ± 4	< 0.001
Calcium (g/1000 kcal)[b]	409 ± 127	421 ± 136	402 ± 116	410 ± 116	405 ± 137	0.395	451 ± 136	470 ± 153	459 ± 131	443 ± 126	432 ± 130	0.342
Supplements (%)												
Fish oil	8.2	6.8	7.2	8.2	10.5	0.227	7.2	4.6	6.2	5.4	12.7	< 0.001
Cod liver oil	34.0	30.0	36.2	36.3	33.7	0.145	32.9	34.0	29.3	32.7	35.5	0.489
Vitamin D	3.4	2.3	2.5	4.2	4.7	0.192	3.0	3.5	2.7	2.3	3.5	0.825
Calcium	7.5	5.3	8.0	9.2	7.7	0.199	12.7	10.0	13.5	13.1	14.3	0.493
Food intake (g/1000 kcal)												
Total fish	35 ± 19	15 ± 5	27 ± 3	38 ± 4	61 ± 17	< 0.001	51 ± 25	18 ± 6	35 ± 4	48 ± 5	78 ± 21	< 0.001
Lean fish	14 ± 11	5 ± 4	10 ± 5	15 ± 7	25 ± 14	< 0.001	23 ± 17	7 ± 5	15 ± 7	21 ± 9	38 ± 20	< 0.001
Fatty fish	10 ± 10	3 ± 3	4 ± 4	6 ± 5	12 ± 10	< 0.001	15 ± 13	3 ± 4	5 ± 5	8 ± 7	14 ± 12	< 0.001
Vegetables	121 ± 77	102 ± 74	112 ± 73	131 ± 78	141 ± 78	< 0.001	118 ± 75	96 ± 74	119 ± 71	122 ± 66	136 ± 82	< 0.001
Fruit and berries	135 ± 83	133 ± 94	136 ± 81	137 ± 78	135 ± 80	0.736	152 ± 102	142 ± 108	162 ± 109	155 ± 86	150 ± 101	0.532
Meat	56 ± 23	54 ± 24	56 ± 22	57 ± 22	58 ± 24	0.018	40 ± 20	34 ± 18	40 ± 19	43 ± 20	42 ± 20	< 0.001
Milk products	134 ± 99	140 ± 112	136 ± 94	137 ± 96	124 ± 93	0.033	192 ± 117	216 ± 136	204 ± 116	187 ± 108	162 ± 99	< 0.001
Egg	9 ± 6	8 ± 6	8 ± 5	8 ± 6	9 ± 6	0.006	10 ± 7	9 ± 7	10 ± 8	9 ± 7	10 ± 8	0.377

The values represented mean ± SD or %. *p* for trend: linear regression for continuous variables and logistic regression for dichotomous variables

[a]Sum of eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)

[b]Total intake from diet and supplements

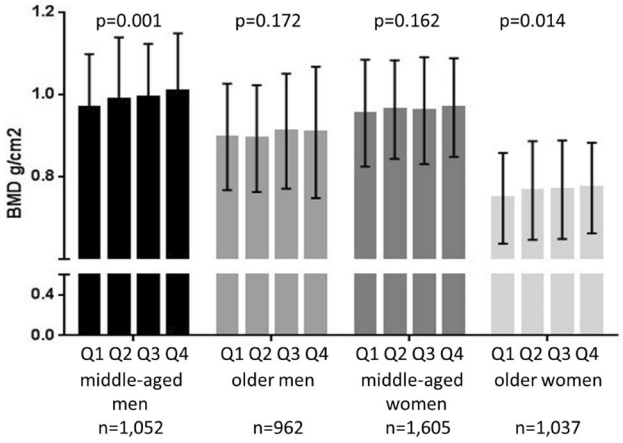

Fig. 2 Femoral neck BMD (g/cm^2) by quartiles (Q1–Q4) of total fish intake in middle-aged (46–49 years) and older (70–74 years) men and women in the Hordaland Health Study

measured BMD was higher in the Americans. This demonstrates the complexity of investigating the association between foods and BMD.

Whereas most studies have a cross-sectional design, there are two studies investigating fish intake and bone loss over time [20, 39]. These studies reported a protective effect of high fish consumption on bone loss, with significant associations for dark fish and tuna in the Framingham study [20], and total fish intake in the Chinese study [39]. Longitudinal studies on the association of fish consumption and BMD may be even more relevant, as bone mass changes throughout life. Thus, nutritional intake including low intake of fish can be regarded as a modifiable risk factors for osteoporosis.

The association between fish intake and BMD has also been analyzed applying a dietary pattern approach. In these analyses, fish consumption is regarded as part of a healthy dietary pattern and was associated with BMD in Australian men and women > 50 years [40] and with Japanese premenopausal women [41], but not in older women in a Finnish study [42].

Possible mechanisms

Fish is a source of nutrients that have been associated with higher BMD, in particular protein [19], n3 PUFAs [16] and vitamin D [43]. A recent meta-analysis and systematic review showed that a higher protein intake was associated with higher BMD at most bone sites (albeit non-significant at most sites) and less bone loss over time [19]. However, it is difficult to translate this into dietary recommendations as 'higher protein' intake was defined differently in the studies included. The assumption that protein intake is important

Table 4 Unstandardized B coefficient for femoral neck BMD by intake of total fish, lean fish, and fatty fish obtained by multiple linear regression models in the middle-aged (46–49 years) and older (70–74 years) men and women in the Hordaland Health Study

	Model 1[a]		Model 2[b]	
	Unstandardized B coefficient (95% CI)	*p*	Unstandardized B coefficient (95% CI)	*p*
Middle-aged				
Men ($n = 1052$)				
Total fish (50 g/1000 kcal)	0.029 (0.007, 0.051)	0.011	0.006 (−0.016, 0.027)	0.612
Lean fish (50 g/1000 kcal)	0.035 (−0.007, 0.076)	0.099	0.007 (−0.033, 0.048)	0.714
Fatty fish (50 g/1000 kcal)	0.043 (0.003, 0.082)	0.033	0.013 (−0.025, 0.051)	0.386
Women ($n = 1605$)				
Total fish (50 g/1000 kcal)	0.010 (−0.006, 0.026)	0.204	0.006 (−0.010, 0.021)	0.475
Lean fish (50 g/1000 kcal)	−0.006 (−0.033, 0.022)	0.680	−0.009 (−0.036, 0.017)	0.484
Fatty fish (50 g/1000 kcal)	0.022 (−0.008, 0.052)	0.144	0.017 (−0.011, 0.046)	0.236
Older				
Men ($n = 962$)				
Total fish (50 g/1000 kcal)	0.015 (−0.003, 0.003)	0.094	0.002 (−0.016, 0.020)	0.842
Lean fish (50 g/1000 kcal)	0.010 (−0.017, 0.037)	0.460	−0.005 (−0.032, 0.022)	0.703
Fatty fish (50 g/1000 kcal)	0.007 (−0.028, 0.043)	0.680	−0.001 (−0.037, 0.035)	0.529
Women ($n = 1037$)				
Total fish (50 g/1000 kcal)	0.018 (0.004, 0.032)	0.014	0.018 (0.003, 0.032)	0.017
Lean fish (50 g/1000 kcal)	0.018 (−0.003, 0.039)	0.100	0.026 (0.004, 0.048)	0.021
Fatty fish (50 g/1000 kcal)	0.024 (−0.002, 0.051)	0.072	0.014 (−0.014, 0.042)	0.337

[a]Adjusted for total energy intake

[b]Adjusted for total energy intake (cont.), BMI (cont.), physical activity score (none/< 1 h/week/1–2 h/week/≥ 3 h/week), cotinine > 85 nmol/L (yes/no), alcohol consumption (none/low/moderate/high)

for maintaining or even increasing BMD is based on the fact that 50% of bone tissue is made up of proteins [44]. Despite an average protein intake in the present study in accordance with the recommendations, about one-third of the older women consumed less than 0.8 g protein/kg body weight per day (Table 2), which is the recommended amount of protein in the Nordic nutritional recommendations 2012 (NNR 2012) [32]. However, even NNR 2012 states that this needs more investigation, and other societies such as the European Society for Clinical Nutrition and Metabolism [45] have recommended higher protein intake for older persons. Fish is a good source of protein and may contribute to a high protein intake. Historically, a high protein intake was thought to be associated with increased urinary loss of calcium [46]. However, current knowledge does not support an adverse effect of diets with high intake of protein as long as the intake of calcium is adequate [19]. It is also important to have a sufficient energy intake to prevent catabolism of body protein for energy purposes.

The positive association of fish consumption with BMD may be due to the n3 PUFA content of fish [17, 20, 21]. Proposed mechanisms explaining the association between n3 PUFA and BMD include the promotion of calcium absorption from the intestine, effect on osteoblastogenesis and osteoblast activity, reduction in inflammation, and modulation of peroxisome proliferators-activated receptor ɣ [16, 17]. Participants in HUSK consumed about twice the amount of n3 PUFA compared to participants in the previous studies [20, 21] due to fish intake, cod liver oil, and fish oil.

Surprisingly, only lean fish was associated with BMD among the older women. No association was found with fatty fish. Fatty fish and cod liver oil are important dietary sources of vitamin D, which is essential for the intestinal absorption of calcium, and a key factor in maintaining the calcium/phosphate homeostasis in serum. An association between high vitamin D status and high BMD has been reported [47]. Although fish is an important dietary source of vitamin D, synthesis of vitamin D in the skin is probably more essential [48]. However, skin synthesis of vitamin D in Norway at 60° northern latitude is only sufficient during the light period of the year. Thus, many Norwegians rely on nutrient sources of vitamin D a large part of the year. A recent meta-analysis concluded that consumption of a recommended amount of fish (300–400 g per week) was not enough to obtain a sufficient vitamin D status (50 nmol/L of 25OHD) [49]. However, due to the habitual intake of both cod liver oil and fatty fish, Norwegians on average have higher vitamin D intake than most other European populations [50].

Strength and limitations

The strength of this community-based study is the large sample size and inclusion of both women and men at different ages. The study included only Caucasians, a population with a high risk of osteoporosis and fractures. Dietary intake was measured using a validated semi-quantitative FFQ [28]. The fish intake reported in our study was similar to intake data obtained in Norwegian dietary surveys [26], suggesting that the estimate was valid. Due to the habitually high fish consumption, it was possible to investigate lean and fatty fish separately, which has not been done by others. The assessment of several other factors associated with BMD allowed extensive adjustment in the multiple linear regression models.

The cross-sectional design of the analysis is a limitation due to non-causal explanation. In addition, we only had information on dietary intake the year before recruitment, whereas bone mass changes take place throughout a longer period of time. However, dietary habits seldom change rapidly, and the intakes reported are likely to represent habits extended throughout a longer period of life.

Conclusion

The total fish intake, in particular lean fish, was positively associated with BMD in older women. No association was found between fatty fish and BMD. Thus, amount and type of fish consumed should be investigated further in different populations as a possible modifiable factor for BMD.

Acknowledgements The Norwegian Seafood Research Fund (FHF) supported this work through fellowship funding the primary author. The funder had no role in the study design, analysis or interpretation of data, or in the writing of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval HUSK was performed according to the declaration of Helsinki and all participants provided written informed consent. The study protocol was approved by the Data Inspectorate and the Regional Committee for Medical Research Ethics.

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