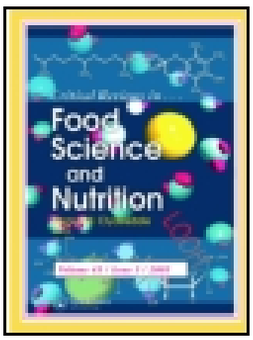

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Dietary intake of fish, n-3 polyunsaturated fatty acids and risk of hip fracture: A systematic review and meta-analysis on observational studies

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ABSTRACT

Previous studies have shown that fish consumption and dietary intake of n-3 polyunsaturated fatty acids (n-3 PUFAs) are associated with hip fracture; however, findings were conflicting. The present review aimed to summarize the current evidence on the association of fish consumption and dietary intake of n-3 PUFAs with hip fracture. The online databases of PubMed, ISI Web of Science, Scopus, ProQuest, Science Direct and Embase were searched until August 2017 for related publications using relevant keywords. To pool data, either a fixed-effects model or random-effects models were used. Cochran's Q tests were used to assess heterogeneity between studies. In total, 10 studies (7 prospective and 3 case-control studies) were included in this systematic review, and 9 studies with total sample size of 292657 participants were included in the meta-analysis. The age of participants was 20 years or older. Combining 8 effect sizes from 4 prospective studies and 2 case-control studies revealed a significant inverse association between fish consumption and risk of hip fracture (pooled effect size: 0.88, 95% CI: 0.79-0.98, P = 0.02). Although this relationship became non-significant in prospective studies, a significant inverse association was found in prospective studies with sample size of 10000 individuals or more, and studies that considered body mass index as a covariate. Furthermore, dietary intake of n-3 PUFAs was inversely associated with risk of hip fracture (pooled effect size: 0.89, 95% CI: 0.80-0.99, P = 0.02). Also, such relationship was seen after excluding one case-control study and combining effect sizes only from prospective studies (pooled effect size: 0.88, 95% CI: 0.80-0.98, P = 0.02). In conclusion, we found that fish consumption and dietary intake of n-3 PUFAs might have protective effects on bone health and decline the risk of hip fracture.

Abbreviations: ALA: α-Linolenic acid; BMD: bone mineral density; DHA: Docosahexaenoic Acid; EPA: Eicosapentaenoic Acid; HR: hazard ratio; ICD: International Statistical Classification of Disease; IL-1: interleukin-1; IL-6: interleukin-6; OR: odds ratio; PUFAs: polyunsaturated fatty acids; RR: relative risk; TNF: tumor necrosis factors

KEYWORDS

Fatty acids; Omega-3; Fishes; hip fracture; meta-analysis

Introduction

Osteoporosis and bone fractures, particularly hip fracture, impose a great burden to healthcare system annually (Center, 2017; Mohd-Tahir and Li, 2017). Recently, the incidence of fractures caused by osteoporosis has been reported high in both men and women (Manthripragada et al., 2015; Litwic et al., 2017). Among bone fractures, hip fracture has been prevalent in older adults at an alarming rate, and associated with high disability, morbidity and mortality (Maghbooli et al., 2017; Majumdar et al., 2017; Maharlouei et al., 2017). Therefore, it is vital to identify appropriate approaches to decrease the risk of hip fracture urgently.

The pathogenesis of hip fracture is complex (Benetos et al., 2007; Luo et al., 2016a). It has been shown that impaired bone strength or decline in bone mineral density (BMD), and trauma from falling lead to hip fracture (Guerado et al., 2016). Earlier studies have shown that physical activity, alcohol consumption, obesity, smoking and dietary intakes are effective factors to BMD, and therefore affect hip fracture (Shin et al., 2013; Karamati et al., 2014; Farsinejad-Marj et al., 2016; Meyer et al., 2016; Ali amd Gibbons, 2017). Among dietary factors, consumption of fruits, vegetables, red meat, dairy products, magnesium and vitamin D can contribute to hip fracture (Benetou et al., 2016; Byberg et al., 2016; Luo et al., 2016b; Laiz et al., 2017). Dietary intake of fish and omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been also investigated in relation to the risk of hip fracture in observational studies (Suzuki et al., 1997; Appleby et al., 2007; Martinez-Ramirez et al., 2007; Orchard et al., 2010; Virtanen et al., 2010;

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Benetou et al., 2011; Farina et al., 2011aa; Virtanen et al., 2012; Fan et al., 2013; Harris et al., 2015); and conflicting results have been reported. Some studies have indicated a significant inverse association between fish consumption and risk of hip fracture (Appleby et al., 2007; Fan et al., 2013), whereas others have reached such association for dietary intake of n-3 PUFAs and α-Linolenic acid (ALA), but not for fish consumption (Farina et al., 2011aa). In addition, some studies have failed to reach any significant association between dietary intake of fish, n-3 PUFAs, and risk of hip fracture (Suzuki et al., 1997; Martinez-Ramirez et al., 2007). Considering the alarming prevalence of hip fracture, high priority should be given to determine contributing factors of bone fractures, particularly dietary factors.

Although there is some evidence on the association between dietary intake of fish, n-3 PUFAs and hip fracture, to the best of researchers' knowledge, no previous study has summarized findings in a review. Therefore, we aimed to summarize the present evidence on the association between dietary intake of fish, n-3 PUFAs and hip fracture.

Methods

This study was performed based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (Moher et al., 2009).

Search strategy

We searched systematically the online databases of PubMed, ISI Web of Science, Scopus, ProQuest, Science Direct and Embase for relevant publications until August 2017. A combination of following keywords including MeSh and text words was used: ("Fatty Acids, Omega-3′ OR "Docosahexaenoic Acid*" OR "Fishes" OR "Fish Oils" OR "Eicosapentaenoic Acid" OR "alpha-Linolenic Acid" OR "Cod Liver Oil" OR "Seafood" OR "Omega 3 Fatty Acid*" OR "n-3 PUFA" OR "n-3 Fatty Acid*" OR "n-3 Polyunsaturated Fatty Acid*" OR "Docosahexaenoic Acid*" OR "fish" OR "Fish Oils" OR "Omega-3′ OR "polyunsaturated FA" OR "EPA" OR "DHA" OR "eicosapentaenoic acid*" OR "alpha-linolenic acid" OR "cod liver oil" OR "seafood" OR "docosapentaenoic acid") AND (Fractures, Bone OR "Osteoporotic Fractures" OR "fractures" OR "fracture" OR "broken bone"). No restriction on time of publication and language was considered. To avoid missing any publication, the reference lists of the relevant papers were reviewed. In the search strategy, unpublished studies were not included. To facilitate the screening process of citations from databases, all literature searches were downloaded into an EndNote library (version X7, for Windows, Thomson Reuters, Philadelphia, PA, USA).

Selection criteria

In this meta-analysis, eligible publications were included based on the following criteria: 1) all studies that were observational with case-control or nested case-control or cohort or cross-sectional design; 2) studies evaluating the association of fish, fish oil consumption or dietary intake of n-3 PUFA [including ALA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] with hip fracture or any fracture (including hip fracture); and 3) those that reported odds ratio (ORs) or relative risks (RRs) or hazard ratios (HRs) along with 95% confidence intervals for hip fracture in relation to fish consumption or dietary intake of n-3 PUFA. If multiple publications drew from the same dataset, only the paper with more complete information or findings was included in the study. In addition, studies that focused on intake of n-3 PUFA supplements were not included in the current review.

Excluded studies

Letters, comments, short communication, reviews, meta-analyses, ecological studies and animal studies were excluded. In our initial search, we found 554 articles. After removing duplicate papers, 467 articles remained. Based on initial title and abstract screening, we excluded 428 studies. Out of 39 remaining articles, 28 of them were excluded because of the following reasons: 1) studies that examined the association between dietary intake of fish or n-3 PUFAs and BMD (n = 13); 2) those that were of clinical trial design (n = 5); 3) publications that assessed the relationship between plasma level of n-3 PUFAs and bone fracture (n = 3); 4) studies that examined the association between dietary factors or fatty acids except fish or n-3 PUFAs and bone fractures (n = 4); and 5) studies that were assessed the association between dietary patterns and bone fractures (n = 3). Of the 11 remaining studies, two had done on the same population (Feskanich et al., 2003; Virtanen et al., 2012). We only included the study with the larger follow-up in the analysis to avoid double-counting data (Virtanen et al., 2012). Among six selected studies which investigated the association between dietary intakes of n-3 PUFAs and risk of hip fracture, three of them did not report separate data for n-3 PUFAs intake from supplements (Orchard et al., 2010; Harris et al., 2015; Martinez-Ramirez et al., 2007); however, they reported that a few number of participants consumed n-3 PUFA supplements. Other three studies reported that inclusion of the n-3 PUFAs from supplements to dietary intake of n-3 PUFAs or excluding those who used supplements, had no appreciable effect on the associations (Virtanen et al., 2010; Virtanen et al., 2012; Farina et al., 2011a). Therefore, six above-mentioned studies were not excluded. Finally, 10 studies [7 prospective (Appleby et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Benetou et al., 2011; Farina et al., 2011a; Virtanen et al., 2012; Harris et al., 2015), and 3 case-control studies (Suzuki et al., 1997; Martinez-Ramirez et al., 2007; Fan et al., 2013)] were included in this systematic review and meta-analysis. The details of the study selection process are shown in Figure 1.

Data extraction

Study selection and data extraction from each eligible study were done independently by two investigators (OS and SG), and any disagreements were resolved by a third investigator (SS-B). In prospective studies, the presence of participants across categories of fish consumption or dietary intake of n-3 PUFAs in the beginning of study was the key exposure variable. In addition, in mentioned studies, the key outcome variable was incidence of hip fracture during the study. Any reported ORs or HRs or RRs for hip fracture among individuals in the highest category of fish

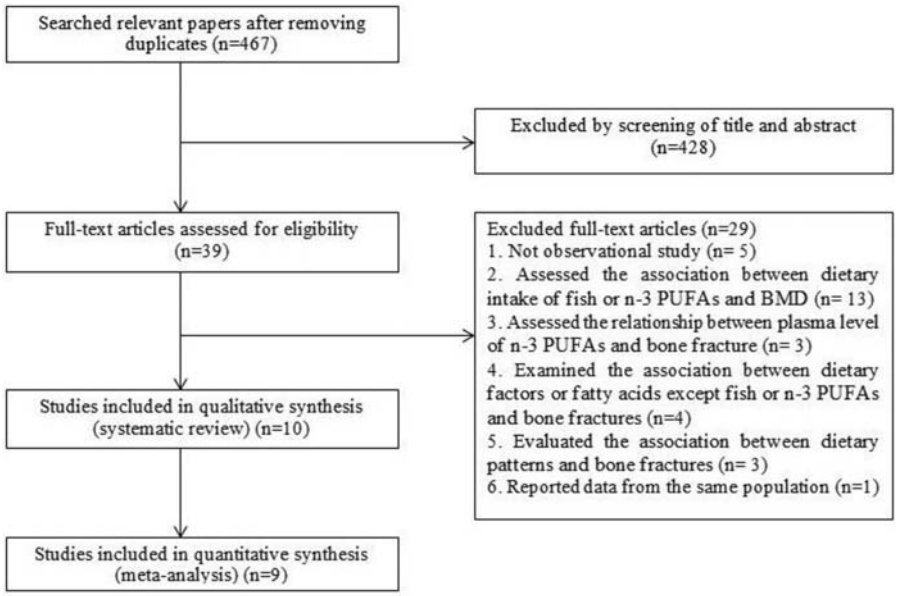

Figure 1. Flow diagram of study selection.

consumption or dietary intake of n-3 PUFAs compared with those in the lowest category, were extracted either in included case-control or prospective studies. In one prospective study, effect sizes for bone fractures were reported for individuals who ate fish (fish eaters) compared with those who did not or ate meat (meat eaters) (Appleby et al., 2007). We considered these effect sizes as same as those reported for the top category of fish consumption compared with the bottom category to include in the meta-analysis. Furthermore, in mentioned prospective study, people with hip fracture due to accident were not excluded, but we could calculate the risk estimate for osteoporotic fractures (including hip fracture) across categories of fish consumption based on available data in the paper to include in the meta-analysis (Appleby et al., 2007). Some prospective studies had reported risk estimates separately for different processed fish including tuna/other fish and fried fish (Virtanen et al., 2010) or for n-3 PUFAs such as ALA, EPA and DHA (Farina et al., 2011a). To include in the meta-analysis, we combined the risk estimates of these items in each study and the combined effect size was used as the RRs for fish and n-3 PUFAs, respectively. However, separate analyses were done based on ALA and EPA plus DHA because of enough studies in this regard. In one prospective study, fish oil consumption was assessed in relation to fractures. We included this study in our meta-analysis, as fish oil is a rich source of n-3 fatty acids (Harris et al., 2015). The following data were extracted from each study: first author, year of publication, country of origin, age range (at study baseline for prospective studies) and mean (for case-control studies), gender, number of subjects (cases, controls or cohort size), duration of follow-up, and person-year for prospective studies, methods used for assessing dietary intakes and hip fracture and statistical adjustment for confounding variables.

Assessment of study quality

The quality of prospective studies was assessed using the Newcastle Ottawa Scale (NOS) which was designed for prospective studies (Wells et al., 2000). Based on this scale, a maximum of nine points can be awarded to each prospective study: four for selection, two for comparability, and three for assessment of outcomes (nine represented the highest quality). Another kind of NOS, designed for case-control studies, was used to assess the quality of included case-control studies (Wells et al., 2000) which considered a maximum of ten points to each case-control study: four for selection, two for comparability, and four for exposure assessment. Any discrepancies were resolved by discussion. In the current review, studies were considered high quality if they scored 6 or more based on the NOS (Supplemental Table 1).

Statistical analysis

In the current meta-analysis, log RRs and their standard errors were calculated using ORs, RRs and HRs and their 95% confidence intervals reported for hip fracture in relation to intake of fish or n-3 PUFAs. The overall effect size was calculated using fixed-effects models. If there was between-study heterogeneity, we also applied random-effects models (DerSimonian–Laird), which take between-study variation into account. To assess between-study heterogeneity, Cochran's Q test and I^2 were used. In the current study, between-study heterogeneity was considered as I^2 values of 50% or more (Green and Higgins, 2008). In addition, we conducted subgroup analysis according to the predefined criteria including gender (males, females, both genders), follow-up duration (less than 10 years and 10 years or above), sample size (less than 10000 people and 10000 people or more), methods used to assess bone fractures (X-ray, self-reported, hospital records) and adjustment for BMI (adjusted and non-adjusted) to find probable sources of heterogeneity. However, due to limited number of case-control studies, we performed subgroup analysis only on the prospective studies. These analyses were done based on fixed-effects models. Furthermore, we did sensitivity analyses to find dependency of the overall estimate on the effect size from a single study. If

the overall estimate was depend on effect size from a study, data were re-analysed by excluding that study. Potential publication bias was assessed by visual inspection of funnel plots and also using two formal tests of Begg and Egger. Statistical analyses were done using Stata, version 11.2 (Stata Corp, College Station, TX). P values were considered significant at the level of < 0.05.

Results

Findings from systematic review

In the current systematic review, 10 studies[7 prospective (Appleby et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Benetou et al., 2011; Farina et al., 2011a; Virtanen et al., 2012; Harris et al., 2015) and 3 case-control studies (Suzuki et al., 1997; Martinez-Ramirez et al., 2007; Fan et al., 2013)]were included. Characteristics of these studies are briefly presented in Tables 1 and 2. The sample size of the included studies ranged from 334 to 136848 individuals, and in total, 321779 individuals, aged 20 years or older, were included in the current systematic review and meta-analysis. The articles have been published between 1997 and 2015. Of the 10 included studies, 4 were conducted in the US (Orchard et al., 2010; Virtanen et al., 2010; Farina et al., 2011a; Virtanen et al., 2012;), 4 in Europe (Appleby et al., 2007; Benetou et al., 2011; Harris et al., 2015; Martinez-Ramirez et al., 2007), and 2 in Asia (Suzuki et al., 1997; Fan et al., 2013). One study was done on women (Orchard et al., 2010), and the rest of included studies were conducted on both gender. However, in 3 studies, statistical analyses were separately done for men and women (Virtanen et al., 2010; Harris et al., 2015; Fan et al., 2013). In prospective studies, the mean duration of follow-up ranged from 5.2 to 24 years.

Fish consumption or dietary intake of n-3 PUFAs had been assessed by food frequency questionnaire (FFQ) in 9 studies (Appleby et al., 2007; Martinez-Ramirez et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Benetou et al., 2011; Farina et al., 2011a; Virtanen et al., 2012; Fan et al., 2013; Harris et al., 2015) and Mediterranean Osteoporosis Study (MEDOS) questionnaire, designed by World Health Organization (WHO) to identify putative risk factors for hip fractures, in one study (Suzuki et al., 1997). In MEDOS questionnaire, fish consumption was weekly assessed by a question with two choices (> 4 or < 2 time/week). Dietary intakes of participants were collected by self-reported questionnaire in 6 studies (Appleby et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Farina et al., 2011a; Virtanen et al., 2012; Harris et al., 2015), interviewer-administered questionnaire in 3 studies (Suzuki et al., 1997; Martinez-Ramirez et al., 2007; Fan et al., 2013), and both type of questionnaires in one study (Benetou et al., 2011). In addition to the assessment of dietary intakes at baseline of the studies, one prospective study had repeated this assessment during the follow-up period (Virtanen et al., 2012). However, all included studies, even those with repeated measurement of exposure, had categorized participants based on the baseline values of fish consumption or dietary intake of n-3 PUFAs.

Four studies had assessed bone fracture in relation to fish consumption (Suzuki et al., 1997; Appleby et al., 2007; Benetou et al., 2011; Fan et al., 2013), one in relation to fish oil consumption (Harris et al., 2015), 2 in relation to dietary intake of n-3 PUFAs (Martinez-Ramirez et al., 2007; Orchard et al., 2010), and 3 other studies in relation to both fish consumption and dietary intake of n-3 PUFAs (Virtanen et al., 2010; Farina et al., 2011a; Virtanen et al., 2012). One prospective study had reported risk estimates separately for different processed fish including tuna/other fish and fried fish (Virtanen et al., 2010). We combined the risk estimates of these items in this study and the combined effect size was considered as the RRs for total fish to include in the meta-analysis. Of 5 studies assessing dietary intake of n-3 PUFAs, 3 assessed ALA (Orchard et al., 2010; Farina et al., 2011a; Virtanen et al., 2012), 4 evaluated EPA+DHA (Orchard et al., 2010; Virtanen et al., 2010; Farina et al., 2011a; Virtanen et al., 2012), one assessed EPA and DHA separately (Farina et al., 2011a) and 3 assessed the dietary intake of total n-3 PUFAs (Martinez-Ramirez et al., 2007; Orchard et al., 2010; Virtanen et al., 2012). In one study without any effect size for total n-3 PUFAs, we combined the risk estimates of ALA and EPA+DHA to obtain the RR for total n-3 PUFAs (Farina et al., 2011a). From 10 included studies, 7 reported risk estimates for hip fracture (Suzuki et al., 1997; Orchard et al., 2010; Virtanen et al., 2010; Benetou et al., 2011; Farina et al., 2011a; Virtanen et al., 2012; Fan et al., 2013) and 3 for any or osteoporotic fractures in relation to dietary intake of fish or n-3 PUFAs Appleby et al., 2007; Martinez-Ramirez et al., 2007; Harris et al., 2015). Data on hip fracture were presented separately in the studies that evaluated any or osteoporotic fractures and therefore, these studies were also included in the systematic review. In the current study, osteoporotic fracture was considered as any fracture. Data about bone fractures had been collected using hospital or medical records in 5 studies (Suzuki et al., 1997; Martinez-Ramirez et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Harris et al., 2015) [two based ICD-9 (Virtanen et al., 2010) or -10 (Harris et al., 2015)], self-reported questionnaire in 2 studies (Appleby et al., 2007; Virtanen et al., 2012), both medical records and self-reported questionnaire in one study (Benetou et al., 2011), and both medical records and interviewer-administered questionnaire in one study (Farina et al., 2011a). Furthermore, fractures had been confirmed using radiograph or x-ray in one study (Fan et al., 2013).

All studies included in the current systematic review had presented adjusted ORs, RRs and HRs and 95% CI for the association of fish consumption or dietary intake of n-3 PUFAs with hip fracture. Eight out of the 10 studies, had reported energy-adjusted (Appleby et al., 2007; Martinez-Ramirez et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Benetou et al., 2011; Farina et al., 2011a; Virtanen et al., 2012; Fan et al., 2013) and 6 had presented BMI-adjusted effect sizes (Suzuki et al., 1997; Appleby et al., 2007; Martinez-Ramirez et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Benetou et al., 2011; Farina et al., 2011a; Virtanen et al., 2012; Fan et al., 2013). Of 7 studies which assessed the association between fish consumption and bone fractures, 7 categorized participants based on the frequency of fish consumption (Suzuki et al., 1997; Virtanen et al., 2010; Farina et al., 2011a; Virtanen et al., 2012; Harris et al., 2015), one based on the quartiles of fish consumption (Fan et al., 2013) and one other study compared fish eaters

Table 1. Risk of hip fracture across categories of fish consumption or dietary intake of n-3 PUFA based on included prospective studies.

Authors (year)	Country	Age range (y)	Gender	Sample size	Cases	Follow-up (y)	Person-Year	Exposure	Exposure assessment	Outcome	Outcome assessment	Comparison	OR, RR or HR (95%CI)	Adjustment[†]
Orchard et al., 2010	USA	50-79	F	136848	1638	7.8		n-3 PUFA ALA EPA+DHA	FFQ: Self-reported	Hip fracture	Blinded clinician using medical records	Q4 vs. Q1	HR: 1.00 (0.84–1.18) HR: 1.03 (0.87–1.21) HR: 0.86 (0.72–1.01)	1,3,4,6,7,8,9,10,11,20,23,24, 25,26,27,28,32,33,34,35, 39,40,41
Benetouet al., 2011	Europe	60 y or more	M/F	29122	275	8	243330	Fish	FFQ: Self-reported or interview	Hip fracture	Self-reported or medical records	Per one quintile increase	HR: 0.93 (0.85-1.02)	1,2,3,10,12,13,32,31,35,41
Appleby et al., 2007	UK	20 y or more	M/F	24150	1154	5.2		Fish	FFQ: Self-reported	Any fracture	Self-reported	Fish vs. meat eater	RR: 0.83 (0.72-0.97)	1,4,12,17,24,32,40,41
Farina et al., 2011	USA	50 y or more	M/F	904	98	M: 10.4 F: 12.7	M: 6467 F: 3522	n-3 PUFA ALA EPA DHA EPA+DHA	FFQ: Self-reported	Hip fracture	Interview and medical records	Q4 vs. Q1	HR: 0.65 (0.46-0.90) HR: 0.46 (0.26-0.83) HR: 0.80 (0.44-1.47) HR: 0.74 (0.42-1.31) HR: 0.83 (0.47-1.46)	1,2,10,12,13,17,20,23,24, 32,41
								Fish				> 3 vs. < 1 serv/wk	HR: 0.66 (0.38-1.17)	
Virtanen et al., 2010	USA	65 y or more	M/F	5045	505	11.1		EPA+DHA Tuna/other fish Fried fish Total fish	FFQ: Self-reported	Hip fracture	Medical records based ICD-9	> 0.47 vs. < 0.09 g/d ≥ 3 serv/w vs. < 1 serv/m ≥ 1 serv/w vs. < 1 serv/m	HR: 0.98 (0.71–1.36) HR: 1.23 (0.83–1.84) HR: 1.16 (0.91–1.49) HR: 1.18 (0.96–1.45)	1,2,3,5,10,11,14,15,16, 17,32,41
Virtanen et al., 2012	USA	30-75	M	46476	529	24	409601	n-3 PUFA ALA EPA+DHA Fish	FFQ: Self-reported	Hip fracture	Self-reported	Q5 vs. Q1 ≥ 5 serv/w vs. < 1 serv/m	RR: 0.96 (0.71-1.31) RR: 1.29 (0.96-1.72) RR: 0.83 (0.60-1.15) RR: 0.72 (0.42, 1.25)	1,12,13,14,17,18,20,21,22,23, 24,29,30,32,36,37,38,41
			F	75878	1051	24		n-3 PUFA ALA EPA+DHA Fish	FFQ: Self-reported	Hip fracture	Self-reported	Q5 vs. Q1 ≥ 5 serv/w vs. < 1 serv/m	RR: 0.86 (0.70-1.06) RR: 0.95 (0.78-1.15) RR: 1.06 (0.84-1.34) RR: 0.95 (0.55, 1.63)	
Harris et al., 2015	Iceland	66-96	M	465	133	7		Fish oil	FFQ: Self-reported	Any fracture	Medical records based ICD-10	daily vs. never	0.70 (0.41, 1.19)	1,3,32,10,11,24,28,35,41
			F	648	265	7		Fish oil	FFQ: Self-reported	Any fracture	Medical records based ICD-10	daily vs. never	0.78 (0.53, 1.13)	

Abbreviation: PUFAs: polyunsaturated fatty acids, ALA: α-Linolenic acid, EPA: Eicosapentaenoic Acid, DHA: Docosahexaenoic Acid, OR: odds ratio, RR: relative risk, HR: hazard ratio, Q: quartile or quintile

[†]Age (1), gender (2), education (3), marital status (4), race (5), ethnicity (6), family history of fracture (7), fracture on or after age 55 y (8), number of falls in past year (9), height (10), weight (11), BMI (12), intake of energy (13), protein (14), fruits (15), tuna/other fish or fried fish (16), alcohol (17), tea (18), caffeine (19), calcium (20), retinol (21), vitamin K (22) and D (23), use of hormone therapy (24), antianxiety (25) or antidepressant (26) medication, bisphosphonate (27), corticosteroid (28) and diuretic (29) drugs, multivitamin (30) and supplement (31) use, smoking (32), history of arthritis (33), depression (34), diabetes (35), osteoporosis (36), CVD (37) and cancer (38), general health assessment (39), parity (40), physical activity (41).

Table 2. Risk of hip fracture across categories of fish consumption or dietary intake of n-3 PUFA based on included case-control studies.

Authors (year)	Country	Gender	Cases (n)	Controls (n)	Age mean (cases)	Age mean (controls)	Exposure	Exposure assessment	Outcome	Outcome assessment	Comparison	OR, RR or HR (95%CI)	Matching factors	Adjustment[†]
Fan et al., 2013	China	M	148	148	70.03	69.49	Fish	FFQ: Interview	Hip fracture	X-ray	Q4 vs. Q1	OR: 0.41 (0.07–2.43)	Age	2,3,4,5,9,10,11,12, 14,15,16,17,18,19,21, 22,23,24,25,29,30,32
		F	433	433	71.37	71.39	Fish	FFQ: Interview	Hip fracture	X-ray	Q4 vs. Q1	OR: 0.42 (0.22–0.78)	Age	
Martinez-Ramirez et al., 2007	Spain	M/F	167	167	73.2	71.2	n-3 PUFA	FFQ: Interview	Any fracture	Hospital records	Q4 vs. Q1	OR: 1.27 (0.47–3.39)	Age, gender	1,15,29,31,32, 33,36,37,38
Suzuki et al., 1997	Japan	M/F	249	498	78.6	78.3	Fish	MEDOS questionnaire: Interview	Hip fracture	Hospital records	$>$ 4 vs. $<$ 2 time/w	0.70 (0.41–1.21)	Age, gender, ethnicity, residential area	6,7,8,9,13,14,20,24, 26,27,28,32,34,35,39

Abbreviation: PUFAs: polyunsaturated fatty acids, OR: odds ratio, RR: relative risk, HR: hazard ratio, Q: quartile, MEDOS: Mediterranean Osteoporosis Study

[†]Age (1), education (2), marital status (3), family history of fracture (4), smoking (5), sun exposure (6), immobilized condition (7), type of bedding (8), occupation (9), income (10), social status (11), house orientation (12), living area (13), BMI (14), intake of energy (15), cereals (16), soybeans (17), vegetables (18), fruits (19), fish (20), meats (21), poultry (22), nuts (23), dairy products (24), tea (25), green tea (26), alcohol (27), coffee (28) and calcium (29), use of multivitamin (30), serum level of vitamin C (31), physical activity (32), underlying disease (33), history of diabetes (34) and stroke (35), number of stairs at home (36), functional status (37), ear disability (38), sleep disturbance (39).

with meat eaters (Appleby et al., 2007). In addition, among 5 studies that classified individuals based on n-3 PUFAs, 3 reported the risk of bone fractures across quartiles of n-3 PUFAs (Martinez-Ramirez et al., 2007; Orchard et al., 2010; Farina et al., 2011a), one assessed this risk across quintiles of n-3 PUFAs (Virtanen et al., 2012), and remained study considered other cut-off points for n-3 PUFAs (Virtanen et al., 2010). In addition to mentioned analyses, in one prospective study, the risk of hip fracture was reported per one quintile increase in the fish consumption (Benetou et al., 2011). All studies were of high quality based on the NOS. Cases of all case-control studies included in our systematic review were inpatient (Suzuki et al., 1997; Martinez-Ramirez et al., 2007; Fan et al., 2013). However, controls were inpatient or outpatient. Among 3 case-control studies, cases and controls were matched based on age in all studies and both age and gender in 2 studies (Suzuki et al., 1997; Martinez-Ramirez et al., 2007).

In the current systematic review, 3 out of 7 included studies showed a significant (Fan et al., 2013; Appleby et al., 2007) or marginally significant (Benetou et al., 2011) inverse association between fish consumption and the risk of hip fracture. In addition, 2 studies reported a significant (Farina et al., 2011a) or marginally significant association (Orchard et al., 2010); with EPA plus DHA (Orchard et al., 2010) and one other study with both total n-3 PUFAs and ALA (Farina et al., 2011a).

Findings from meta-analysis

Of 10 studies, 9 studies including 6 prospective studies (Appleby et al., 2007; Orchard et al., 2010; Virtanen et al., 2010; Farina et al., 2011a; Virtanen et al., 2012; Harris et al., 2015) and 3 case-control studies (Orchard et al., 2010; Martinez-Ramirez et al., 2007; Fan et al., 2013) were included in the systematic review. One prospective study was excluded because risk estimates for fractures had been reported based on one quintile increase in fish consumption (Benetou et al., 2011), while other studies presented risk estimates across categories of fish consumption. Out of 9 studies included in the meta-analysis, 3 reported risk estimates for osteoporotic or any fractures, not just hip fracture (Appleby et al., 2007; Harris et al., 2015; Martinez-Ramirez et al., 2007). Given the hip fracture was a part of any fractures in the abovementioned studies; we did meta-analysis with and without considering these three studies. The total sample size of studies included to the current meta-analysis was 292657 participants. Among them, 4651 individuals had hip fracture and 1719 people were cases of any fractures (including hip or other bone sites).

Overall and subgroup analysis (based on study design) on the association between fish consumption and risk of hip fracture was presented in Figure 2. Combining 8 effect sizes from 4 prospective studies and 2 case-control studies revealed a significant inverse association between fish consumption and risk of hip fracture (pooled effect size: 0.88, 95% CI: 0.79-0.98, P = 0.02). However, a between-study heterogeneity was found (I^2 = 57.9%; $P_{heterogeneity}$ = 0.02). When we did subgroup analysis based on study design, such findings were also seen for case-control studies (pooled effect size: 0.56, 95% CI: 0.37-0.83, P = 0.004), while became non-significant for prospective studies (pooled effect size: 0.91, 95% CI: 0.82-1.02, P = 0.11) (I^2 = 58.1%; $P_{heterogeneity}$ = 0.04). The overall association between fish consumption and the risk of hip fracture attenuated and became marginally significant when we did random-effects analysis (pooled effect size: 0.80, 95% CI: 0.64-1.01, P = 0.05) (Supplemental Figure 1). We repeated analyses after excluding one prospective study on any fracture and keeping only studies with hip fracture as the main outcome. After this exclusion, the association between fish consumption and risk of hip fracture in total studies (pooled effect size: 0.94, 95% CI: 0.80-1.10, P = 0.46) and prospective studies (pooled effect size: 1.04, 95% CI:

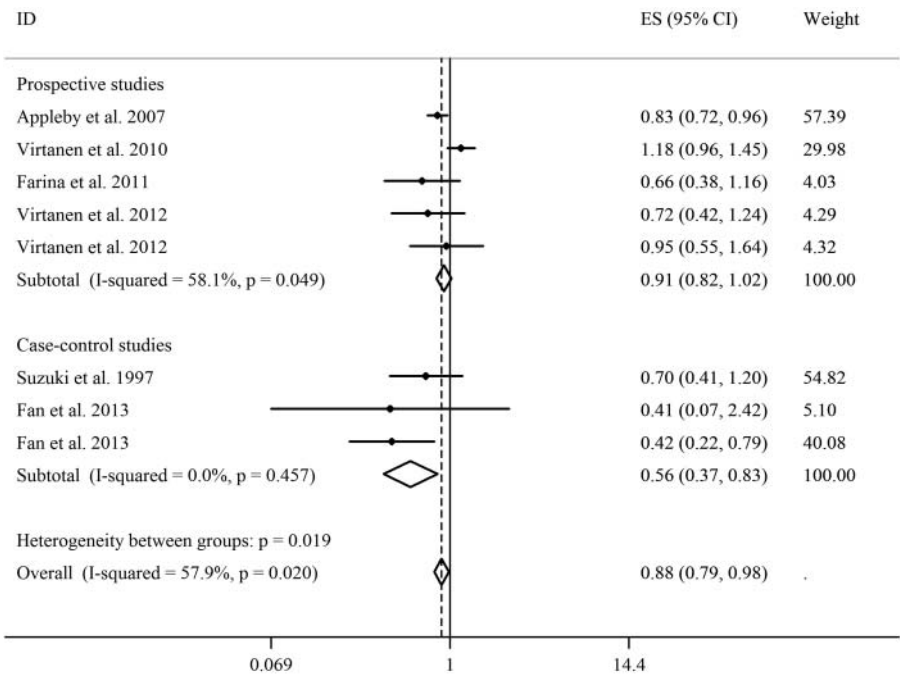

Figure 2. Forest plot for the association between fish consumption and risk of fractures (including hip and any fractures) using a fixed-effects model.

0.87-1.24, P = 0.65) became non-significant (Supplemental Figure 2). However, between-study heterogeneity was disappeared by subgroup analysis based on study design. Such findings were also observed in random-effects model (pooled effect size: 0.76, 95% CI: 0.55-1.05, P = 0.09) (Supplemental Figure 3).

To find other sources of heterogeneity in addition to study design, we performed subgroup analysis on prospective studies based on gender (males, females, both genders), follow-up duration (less than 10 years, 10 years or above), sample size (less than 10000 people, 10000 people or more), methods used to assess bone fractures (X-ray, self-reported, hospital records) and adjustment for BMI (adjusted, non-adjusted) (Table 3). We did not exclude studies on any fractures in mentioned analysis. Based on these analyses, duration of follow-up and adjustment for BMI could explain between-study heterogeneity. In addition, we found a significant inverse association between fish consumption and risk of hip fracture for studies with sample size of 10000 individuals or more (pooled effect size: 0.83, 95% CI: 0.72-0.95, P = 0.008), those that assessed bone fractures using self-reported questionnaires (pooled effect size: 0.83, 95% CI: 0.72-0.95, P = 0.008) and studies that considered BMI as a covariate in the analysis (pooled effect size: 0.82, 95% CI: 0.72-0.94, P = 0.004). However, between-study heterogeneity was significant when the subgroup analysis was done based on methods used to assess bone fractures and BMI-adjustment.

The association between dietary intake of n-3 PUFAs and risk of hip fracture was shown in Figure 3. We pooled 7 effect sizes from 4 prospective and one case-control studies and found an inverse association between dietary intake of n-3 PUFAs and hip fracture risk (pooled effect size: 0.89, 95% CI: 0.80-0.99, P = 0.02). No between-study heterogeneity was found (I^2 = 17.3%; $P_{heterogeneity}$ = 0.29). After excluding one case-control study, this relationship remained significant (Supplemental Figure 4); such that greater dietary intake of n-3 PUFAs was associated with 12% lower risk for having hip fracture (pooled effect size: 0.88, 95% CI: 0.80-0.98, P = 0.02) (I^2 = 25.9%; $P_{heterogeneity}$ = 0.24). In addition, excluding 2 studies on any fractures and keeping only the prospective studies with hip fracture as the main outcome, the relationship became marginally significant (pooled effect size: 0.90, 95% CI: 0.81-1.01, P = 0.08) without between-study heterogeneity (I^2 = 44.8%; $P_{heterogeneity}$ = 0.14) (Supplemental Figure 5). Due to no between-study heterogeneity in mentioned relationships, random-effects analysis were not conducted (I^2 < 50%). Findings of subgroup analysis on prospective studies (including hip and any fractures) revealed a significant inverse association between dietary intake of n-3 PUFAs and risk of fracture for studies that had less than 10000 people sample size (pooled effect size: 0.70, 95% CI: 0.56-0.88, P = 0.002), those with 10-year duration of follow-up or above (pooled effect size: 0.83, 95% CI: 0.72-0.97, P = 0.02) as well as studies that adjusted BMI in the analysis (pooled effect size: 0.83, 95% CI: 0.72-0.97, P = 0.02) (Table 3). In mentioned subgroup analyses, between-study heterogeneity was completely disappeared.

For studies that evaluated dietary intake of ALA, we found that combining 4 effect sizes from 3 prospective studies (all assessed only hip fracture) presented no significant relationship between dietary intake of ALA and risk of hip fracture (pooled effect size: 1.01, 95% CI: 0.90-1.13, P = 0.92) (Figure 4). However, between-study heterogeneity was significant (I^2 = 70.6%; $P_{heterogeneity}$ = 0.01). This relationship remained non-significant when we did random-effects meta-analysis (pooled effect size: 0.97, 95% CI: 0.76-1.22, P = 0.77) (Supplemental Figure 6). We conducted subgroup analysis to find sources of heterogeneity (Table 3). Doing mentioned analysis based on gender and sample size (less than 10000 people, 10000 people or more) showed no between-study heterogeneity. No significant association was found between dietary intake of ALA and risk of fracture according to findings of subgroup analysis.

In terms of dietary intake of EPA plus DHA, we combined 5 effect sizes from 4 prospective studies (all assessed only hip fracture) and found no significant association between dietary intake of EPA plus DHA and risk of hip fracture (Figure 5) (pooled effect size: 0.91, 95% CI: 0.81-1.03, P = 0.12). No between-study heterogeneity was observed (I^2 = 0; $P_{heterogeneity}$ = 0.61). Therefore, random-effects analysis was not done. In addition, findings of subgroup analysis revealed no significant association between dietary intake of EPA plus DHA and hip fracture risk (Table 3). In these analyses, no between-study heterogeneity was seen.

We found no evidence of publication bias based on visual inspection of funnel plots (Supplemental Figure 7) and also according to results of Begg's test (P > 0.44). Based on findings from sensitivity analysis, pooled effect sizes obtained for the association of fish consumption and dietary intake of n-3 PUFAs with risk of hip fracture, was not depend on a particular study or group of studies.

Discussion

In the current study, we found that fish consumption was inversely associated with risk of hip fracture. Such finding was also seen for prospective studies with sample size of 10000 individuals or more, those that assessed bone fractures using self-reported questionnaires and studies that considered BMI as a covariate in the analysis. Furthermore, we found that higher dietary intake of n-3 PUFAs was significantly associated with decreased risk of hip fracture. This significant relationship was also seen for studies that had population with less than 10000 individuals, those with 10-year duration of follow-up or above as well as studies that adjusted BMI in the analysis. However, separate analyses for ALA and EPA plus DHA, as important subgroups of n-3 PUFAs, revealed no significant association. To the best of our knowledge, this is the first study summarizing earlier studies to assess the association between fish consumption and dietary intake of n-3 PUFAs with the risk of hip fracture.

Recently, some studies have assessed the association between dietary intakes and osteoporotic fractures (Jackson et al., 2006; Caire-Juvera et al., 2009; McTiernan et al., 2009; Sahni et al., 2009). It has been shown that fish consumption and dietary intake of n-3 PUFAs might have protective effects against osteoporosis and bone fractures (Appleby et al., 2007; Fan et al., 2013); however, data are conflicting. In the current meta-analysis, we found that fish consumption was inversely associated with hip fracture. In line with our findings, it was reported that higher adherence to a Mediterranean-like diet, rich in fish and sea foods, is associated with lower risk of hip fracture (Byberg et al., 2016). In a case-control study, it has been shown that less

Table 3. Subgroup analysis based on fixed effects models for the association of fish consumption and dietary intake of n-3 PUFAs with risk of hip fracture.

	effect sizes (n)	I^2	Q test	RR (95%CI)	$P_{between}$
Fish					
Overall	5	58.1	0.04	0.91 (0.82–1.02)	
Gender					0.67
Male	1	0	0	0.72 (0.42–1.24)	
Female	1	0	0	0.95 (0.55–1.64)	
Both	3	77.2	0.01	0.92 (0.82–1.04)	
Sample size					0.02
Under 10000 people	2	72.3	0.05	1.10 (0.91–1.34)	
10000 people or more	3	0	0.77	0.83 (0.72–0.95)	
Follow-up					0.05
Under 10 years	1	0	0	0.83 (0.72–0.96)	
10 years or above	4	48.3	0.12	1.04 (0.87–1.24)	
Methods of fracture assessment					0.02
Self-reported questionnaires	3	0	0.77	0.83 (0.72–0.95)	
Medical records	2	72.3	0.05	1.10 (0.91–1.34)	
Adjustment for BMI					0.004
Adjusted effect size	4	0	0.77	0.82 (0.72–0.94)	
Non-adjusted effect size	1	0	0	1.18 (0.96–1.45)	
Total n-3 PUFAs					
Overall	6	25.9	0.24	0.88 (0.80–0.98)	
Gender					0.15
Male	2	1.5	0.31	0.89 (0.68–1.16)	
Female	3	2.9	0.35	0.92 (0.81–1.04)	
Both	1	0	0	0.65 (0.46–0.91)	
Sample size					0.02
Under 10000 people	3	0	0.77	0.70 (0.56–0.88)	
10000 people or more	3	0	0.54	0.94 (0.84–1.07)	
Follow-up					0.29
Under 10 years	3	23.5	0.27	0.94 (0.81–1.09)	
10 years or above	3	33.7	0.22	0.83 (0.72–0.97)	
Methods of fracture assessment					0.92
Self-reported questionnaires	2	0	0.56	0.89 (0.75–1.06)	
Medical records	4	53.1	0.09	0.88 (0.77–1.01	
Adjustment for BMI					0.29
Adjusted effect size	3	33.7	0.22	0.83 (0.72–0.97)	
Non-adjusted effect size	3	23.5	0.27	0.94 (0.81–1.09)	
ALA					
Overall	4	70.6	0.01	1.01 (0.90–1.13)	
Gender					0.007
Male	1	0	0	1.29 (0.96–1.73)	
Female	2	0	0.53	1.00 (0.88–1.13)	
Both	1	0	0	0.46 (0.26–0.82)	
Sample size					0.007
Under 10000 people	1	0	0	0.46 (0.26–0.82)	
10000 people or more	3	32	0.23	1.04 (0.92–1.16)	
Follow-up					0.69
Under 10 years	1	0	0	1.03 (0.87–1.21)	
10 years or above	3	80.1	0.007	0.98 (0.84–1.15)	
Methods of fracture assessment					0.52
Self-reported questionnaires	2	65.9	0.08	1.04 (0.89–1.23)	
Medical records	2	85.4	0.009	0.97 (0.83–1.14)	
Adjustment for BMI					0.69
Adjusted effect size	3	80.1	0.007	0.98 (0.84–1.15)	
Non-adjusted effect size	1	0	0	1.03 (0.87–1.21)	
EPA+DHA					
Overall	5	0	0.61	0.91 (0.81–1.03)	
Gender					0.81
Male	1	0	0	0.83 (0.60–1.15)	
Female	2	50.5	0.15	0.92 (0.81–1.06)	
Both	2	0	0.61	0.94 (0.71–1.25)	
Sample size					0.83
Under 10000 people	2	0	0.61	0.94 (0.71–1.25)	
10000 people or more	3	15.8	0.3	0.91 (0.80–1.03)	
Follow-up					0.33
Under 10 years	1	0	0	0.86 (0.73–1.02)	
10 years or above	4	0	0.63	0.96 (0.82–1.13)	
Methods of fracture assessment					0.4
Self-reported questionnaires	2	30.2	0.23	0.98 (0.81–1.18)	
Medical records	3	0	0.76	0.88 (0.76–1.02)	
Adjustment for BMI					0.49
Adjusted effect size	3	0	0.61	0.91 (0.81–1.03)	
Non-adjusted effect size	2	0	0.48	0.88 (0.76–1.03)	

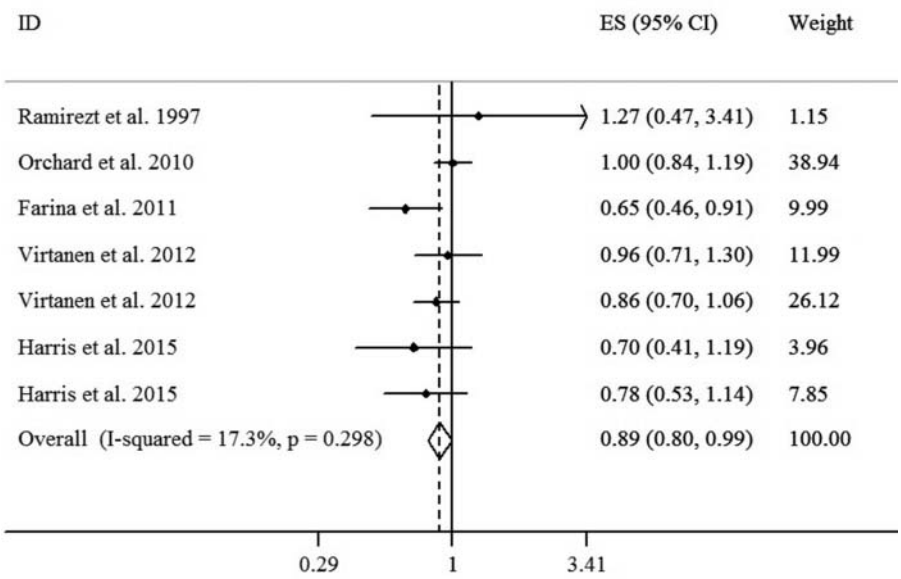

Figure 3. Forest plot for the association between dietary intake of n-3 PUFAs and risk of fractures (including hip and any fractures) using a fixed-effects model.

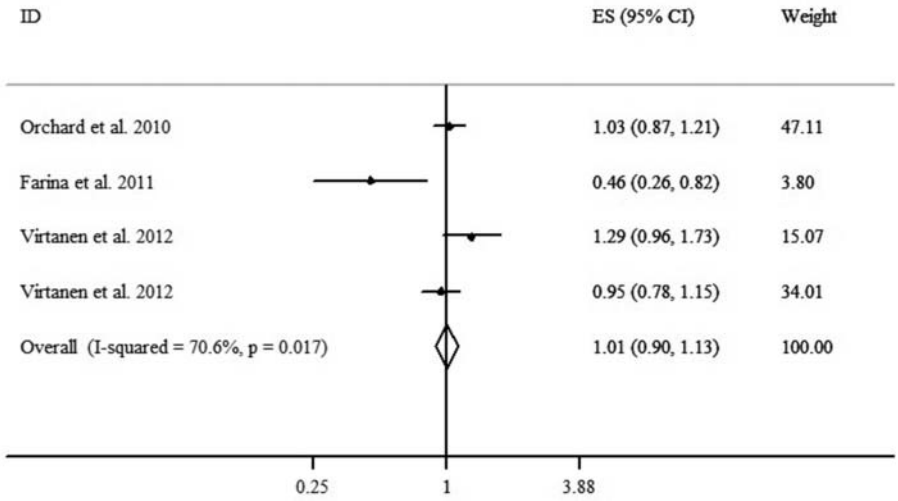

Figure 4. Forest plot for the association between dietary intake of ALA and risk of hip fracture using a fixed-effects model.

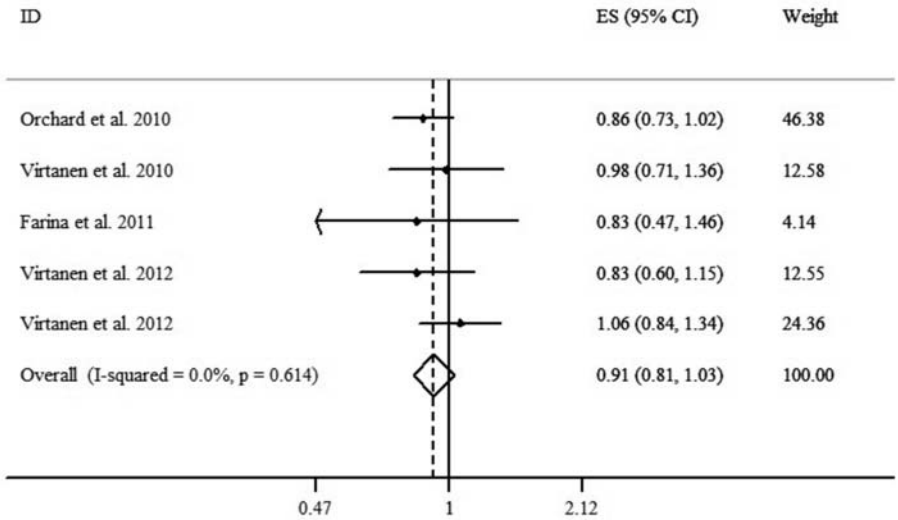

Figure 5. Forest plot for the association between dietary intake of EPA plus DHA and risk of hip fracture using a fixed-effects model.

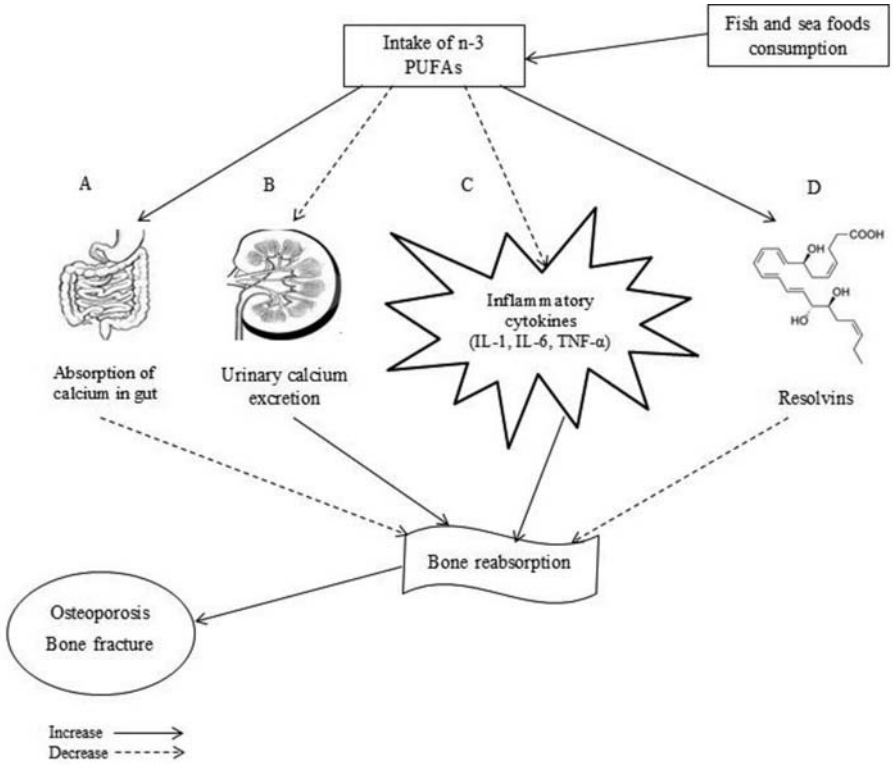

Figure 6. Mechanisms explained the protective effects of fish consumption and dietary intakes of n-3 PUFAs on osteoporosis and bone fracture through; A) increase the absorption of calcium in gut, B) decrease the excretion of calcium in the kidney, C) inhibiting the action of inflammatory cytokines such as IL-1, IL-6 and TNF-α that are known to enhance bone reabsorption and supress bone formation, D) increasing the Resolvins as lipid mediators that inhibit induced bone reabsorption.

frequent consumption of fish is associated with increased risk of osteoporosis (Grgurevic et al., 2010). In addition in Koreans, it was indicated that consumption of fish and shellfish increased BMD (Choi and Park, 2016). In two studies, it have been shown the individuals with higher intake of fish had greater maintenance of femoral neck BMD compared with those with lower intake (Chen et al., 2010; Farina et al., 2011b). In contrast, in a recent study no significant association was shown between fish consumption or dietary pattern rich in fish and femoral, lumbar or total body BMD (Erkkila et al., 2017). In another study, fish consumption was not significantly associated with bone fractures (Benetou et al., 2011). It must be kept in mind that studies which reached no significant association had mostly low sample sizes (Farina et al., 2011a) or different design (Suzuki et al., 1997) than those that reached an inverse relationship (Appleby et al., 2007). In our systematic review, among 4 studies that reached no significant association between fish consumption and hip fracture (Suzuki et al., 1997; Virtanen et al., 2010; Farina et al., 2011a; Virtanen et al., 2012), 3 studies considered BMI as a covariate (Suzuki et al., 1997; Farina et al., 2011a; Virtanen et al., 2012), whereas in all studies with significant or marginally significant inverse association, this adjustment was done (Appleby et al., 2007; Benetou et al., 2011; Fan et al., 2013). In addition, different cut-offs used in included studies might be a reason for a weak association between fish consumption and hip fracture in prospective studies.

In the present study, dietary intake of n-3 PUFAs was inversely associated with hip fracture. In line with our finding, in a review study it was concluded that greater intake of total PUFAs, total n-6 and n-3 fatty acids was associated with higher BMD and reduced risk of fragility fracture (Longo and Ward, 2016). In an experimental study on free-range laying hens, intervention with an omega-3 enhanced diet resulted in a reduction in fracture likely by increasing bone strength (Toscano et al., 2015). In a prospective study with 3-year duration of follow-up, a positive relationship between the dietary PUFAs and BMD at lumbar spine and in total body was reported (Jarvinen et al., 2012). Such relationship was also reported in two cross-sectional studies (Rousseau et al., 2009; Mangano et al., 2014). However, in one mentioned study, the association between n-3 fatty acids and BMD was marginally significant (Mangano et al., 2014). In contrast to our findings, in one study a significant inverse association between dietary intake of PUFAs and BMD was observed (Harris et al., 2015). Based on the cross-sectional design of mentioned study, it cannot confer a causal link between PUFAs and BMD, while prospective studies considered in our systematic review, have appropriate design in this regard. In the current systematic review, 3 from 4 studies that reached no significant association between dietary intake of n-3 PUFAs and risk of hip fracture, had included less than 10000 participants and had not controlled for BMI in the analysis (Martinez-Ramirez et al., 2007; Virtanen et al., 2010; Harris et al., 2015). While out of 2 studies that reached a significant or marginally significant inverse association (Orchard et al., 2010; Farina et al., 2011a), one had a sample size of more than 10000 individuals (Orchard et al., 2010), and one had controlled for BMI (Farina et al., 2011a).

In the current study, obtained association between dietary intake of n-3 PUFAs and risk of hip fracture was independent

of n-3 PUFAs intake from supplements. In six included studies, three of them reported that excluding those who used mentioned supplements did not change the relationships (Virtanen et al., 2010; Virtanen et al., 2012; Farina et al., 2011a). The remaining three studies have been reported that a few number of participants consumed n-3 PUFAs supplements that had no considerable effect on the association between dietary intake of n-3 PUFAs and risk of hip fracture (Orchard et al., 2010; Harris et al., 2015; Martinez-Ramirez et al., 2007).

The effects of fish consumption on bone health might be different compared with intake of n-3 PUFAs from the other dietary sources except fish or sea foods. However, we cannot separate fish consumption from intake of n-3 PUFAs because fish and sea foods are the main sources of long chain n-3 PUFAs. In addition to n-3 PUFAs, fish and sea foods are rich in vitamin D that has long been thought to enhance the beneficial effects of calcium on bone health (Lehmann et al., 2015). Moreover, sea foods contain a high quality proteins requiring for bone turnover (Prester, 2011). Therefore, fish consumption seems to have more beneficial effects on bone health than only intake of n-3 PUFAs. In the current study, the protective effect of fish consumption (pooled effect size: 0.88, 95% CI: 0.79-0.98) on hip fracture was 1% greater than dietary intake of n-3 PUFAs (pooled effect size: 0.89, 95% CI: 0.80-0.99). However, in most of studies which have been considered n-3 PUFAs, dietary intake of these fatty acids was mostly obtained from fish and sea foods.

Osteoporosis or low bone mineral density is the main risk factor increasing the risk of hip fracture (Benetos et al., 2007). Earlier studies have shown that n-3 PUFAs might increase the BMD through several mechanisms. These fatty acids can increase calcium absorption and synthesis of bone collagen (Hankenson et al., 2000; Koren et al., 2014). In addition, n-3 fatty acids have a role in inhibiting bone reabsorption and decreasing urinary calcium excretion (Zwart et al., 2010; Ortiz-Alvarado et al., 2012). One of the most important roles of n-3 fatty acids, particularly long chain n-3 PUFAs, is modulating the action of inflammatory cytokines such as interleukin-1, interleukin-6, and tumour necrosis factor (Pannacciulli et al., 2001; Sun et al., 2003). It has been shown that inflammatory cytokines uncouple bone remodelling by enhancing bone reabsorption and suppressing bone formation (Kawai et al., 2012). Furthermore, n-3 PUFAs gives rise to lipid mediators such as Resolvins that inhibit induced bone reabsorption (Zhang and Spite, 2012; Norling and Perretti, 2013). Fishes and sea foods are one of the n-3 PUFAs sources, and therefore consumption of these foods can have a protective effect against osteoporotic fractures. However, the full effect of fish consumption might not be due to n-3 PUFAs.

The current meta-analysis was the first to assess the association of fish consumption and dietary intake of n-3 PUFAs with hip fracture. However, some limitations should be considered in this study. Some studies had assessed EPA and DHA separately in relation to bone fractures; however, due to limited number of studies, we could not evaluate the separate associations of these fatty acids. Although sub-group analysis was done according to gender, sample size, duration of follow-up, methods used to assess bone fractures and adjustment for BMI, we could not perform these analyses based on other variables including age group and geographical region due to limited number of studies. Adjustment of BMI is necessary for assessing the association between dietary intakes and bone fracture. Among 10 studies included in the current systematic review, only 5 studies had considered BMI as a covariate. In addition, the effect of other influencing factors, particularly energy intake, was not controlled in some included studies. Therefore, adjustment of other variables might attenuate the observed associations. In addition, different statistical analyses were used in included studies to assess relationship between fish consumption or dietary intake of n-3 PUFAs and bone fractures.

Conclusion

We found that higher consumption of fish might be associated with decreased odds for having hip fracture. Such finding was also seen for prospective studies with sample size of 10000 individuals or more and studies that considered BMI as a covariate. Furthermore, dietary intake of n-3 PUFAs was inversely associated with risk of hip fracture. This relationship was also significant in studies with 10-year duration of follow-up or more and those that adjusted BMI in the analysis.

Conflicts of interest

Authors declared no personal or financial conflicts of interest.

Authors' contribution

OS, KD, MN, SG and MK contributed in conception, design, statistical analyses, data interpretation and manuscript drafting. SS contributed in data analysis, data interpretation and manuscript drafting. All authors approved the final manuscript for submission.

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