Efficacy of fish intake on vitamin D status: a meta-analysis of randomized controlled trials[1,2]

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ABSTRACT

Background: It is well known that fish is the major natural source of vitamin D in the diet; therefore, this meta-analysis investigated the influence of fish consumption in randomized controlled trials (RCTs) on serum 25-hydroxyvitamin D [25(OH)D] concentrations.

Objective: A literature search was carried out in Medline, Embase, Web of Science, and the Cochrane Library (up to February 2014) for RCTs that investigated the effect of fish consumption on 25(OH)D concentrations in comparison to other dietary interventions.

Results: Seven articles and 2 unpublished study data sets with 640 subjects and 14 study groups met the inclusion criteria and were included in this meta-analysis. Compared with controls, the consumption of fish increased 25(OH)D concentrations, on average, by 4.4 nmol/L (95% CI: 1.7, 7.1 nmol/L; $P < 0.0001$, $I^2 = 25\%$; 9 studies).The type of the fish also played a key role: the consumption of fatty fish resulted in a mean difference of 6.8 nmol/L (95% CI: 3.7, 9.9 nmol/L; $P < 0.0001$, $I^2 = 0\%$; 7 study groups), whereas for lean fish the mean difference was 1.9 nmol/L (95% CI: −2.3, 6.0 nmol/L; $P < 0.38$, $I^2 = 37\%$; 7 study groups). Short-term studies (4–8 wk) showed a mean difference of 3.8 nmol/L (95% CI: 0.6, 6.9 nmol/L; $P < 0.02$, $I^2 = 38\%$; 10 study groups), whereas in long-term studies (∼6 mo) the mean difference was 8.3 nmol/L (95% CI: 2.1, 14.5 nmol/L; $P < 0.009$, $I^2 = 0\%$; 4 study groups).

Conclusion: As the major food source of vitamin D, fish consumption increases concentrations of 25(OH)D, although recommended fish intakes cannot optimize vitamin D status. *Am J Clin Nutr* 2015;102:837–47.

Keywords: fish intake, meta-analysis, randomized controlled trial, vitamin D, intervention studies, 25(OH)D, vitamin D status

INTRODUCTION

Vitamin D deficiency is a global problem and is associated with an increased risk of cardiovascular diseases (1–4), autoimmune diseases (5), type 1 diabetes (6, 7), osteoporosis (6), and probably various types of cancer (8–10). Although vitamin D is synthesized in the skin on exposure to UV-B radiation, it is not possible to maintain an adequate vitamin D status during winter at high latitudes when UV-B radiation is absent (11). Fish, egg yolk, cheese, and mushrooms are the only dietary sources that contain natural vitamin D (12). Among these, fish has, in general, the highest content of vitamin D (12, 13) and is the major natural food source in many populations within (14–17) and outside of (18, 19) Europe. Other significant food sources are fortified items such as margarine, skimmed milk, and orange juice (20, 21). Although, in general, fish is a good source of vitamin D, there are considerable differences in vitamin D content between different fish species (13, 22). Other important factors are environmental conditions, such as season, and the fat content of the fish (13), but more research is needed in this area.

In observational studies (23, 24) fish consumption was shown to have a beneficial effect on cardiovascular morbidity and mortality, although it must be considered that these health effects could also be due to other constituents present in fish, such as long-chain n–3 PUFAs, amino acids, iodine, or selenium, in addition to vitamin D. The effects of short- to medium-term fish interventions on PUFAs (25–28, 30), blood lipids (25–27, 29–34), vitamin B-12 and selenium status (28, 35), insulin and leptin concentrations (29), eicosanoids and adhesion molecule concentrations (36), heart rate variability (25, 34), and vitamin D status have been investigated in several randomized controlled trials (RCTs)[13] (25–28, 34, 35, 37), but systematic studies of the

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[2] Supplemental Text and Supplemental Tables 1–3 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[13] Abbreviations used: LC-MS/MS, liquid chromatography–tandem mass spectrometry; RCT, randomized controlled trial; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxycholecalciferol.

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extent to which fish consumption may contribute to dietary status or to biomarkers for nutrient uptake are lacking. To the best of our knowledge, the effect of fish consumption on vitamin D status has not been investigated systematically. Because increased vitamin D intake due to regular fish consumption may be one explanation for the beneficial health effects of fish, the aim of this study was to conduct a meta-analysis of RCTs on the effect of fish consumption on serum 25-hydroxyvitamin D [25(OH)D] concentrations as the outcome.

METHODS

Search strategies and data collection

To identify relevant studies, Medline, Embase, Web of Science, and the Cochrane Library databases were searched between January 1950 and 12 February 2014. The following search terms were used: vitamin D, cholecalciferol, ergocalciferol, hydroxycholecalciferols, dihydroxycholecalciferol, calcitriol, 24,25-dihydroxyvitamin D, 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, calcitriol or calcidiol, fishes, seafood, shellfish, clinical trial, and random trial or parallel trial (as shown in **Supplemental Table 1**). Additional studies were identified by manual searches through references or the clinicaltrials.gov database. The search was restricted to studies published in English.

The studies were assessed by 2 independent investigators (UL and JD), taking the inclusion criteria into account. Data on the primary patients were collected by personal communication with the relevant investigators by e-mail. Standard data files were provided for this purpose. Investigators who agreed to collaborate were asked to provide data for each participant, including the measured serum 25(OH)D concentration, the definition of the fish consumption group, age and sex, BMI, and the season in which blood samples were taken.

Study eligibility criteria

Any randomized intervention trial that involved human adults and investigated the effects of fish meals on serum 25(OH)D concentrations was included in the analysis. We excluded studies that used only a food-frequency questionnaire to calculate fish intake and studies with only 1 fish meal/wk as an intervention (38). In addition to studies in healthy participants, those that included patients who had survived a myocardial infarction or overweight subjects were also included in the meta-analysis. Studies that involved children, adolescents, or pregnant or breastfeeding women were excluded. Differences between the extracted studies in daily fish intake, the frequency of meals containing fish, or study duration were not a cause for exclusion.

Data collection

The quality of the included studies was checked manually by careful examination of the original publications. Several studies did not originally intend to evaluate the effect of fish consumption on vitamin D status, and therefore most studies did not adequately report the methods of 25(OH)D measurement or the season of blood collection. Because this meta-analysis was concerned with the effect of real food, the issue of blinding was not applicable to the participants. Indeed, only 1 study (U Lehmann, unpublished data, 2012) was sufficiently blinded to participants, as expected in studies investigating natural food. In most studies, meat was used as the comparator or no food was provided to the participants in the control group. In 2 studies, fish with a low vitamin D content was used as the control intervention (27; U Lehmann, unpublished data, 2012). The accepted quality-control measures, such as the Jadad scale (43) or the CONSORT(Consolidated Standards of Reporting Trials) statement (44), were therefore not appropriate for estimating study quality. The quality of the studies was instead assessed on the basis of compliance, number of dropouts, measurements of the vitamin D content in the fish, season of the intervention, the type of vitamin D analysis, and the type of randomization. One score point was given for each item of information included. Scores of 5–6 denote good quality, 3–4 indicate moderate quality, and 0–2 points denote low quality.

Analysis of the data

Studies were analyzed by using RevMan 5.2, which was provided by the Cochrane Collaboration. After consultation with the relevant authors, we received individual patient data from 6 trials (26–28, 35; U Lehmann, unpublished data, 2012; OA Gudbrandsen, unpublished data, 2014). For each study we recorded the number of subjects and mean (SD) baseline and postintervention 25(OH)D concentrations separately for controls and for the intervention group. The mean change in 25(OH)D was calculated by subtracting the mean baseline 25(OH)D concentration from the mean postintervention 25(OH)D concentration. For calculation of the SD of the change in 25(OH)D we applied a correlation coefficient of 0.82 in the control group and a correlation coefficient of 0.77 in the intervention group. These correlation coefficients were calculated from studies with access to individual data ($n = 6$), according to the Cochrane Handbook (39).

Studies that included >1 intervention group (26–28, 37; OA Gudbrandsen, unpublished data, 2014) were treated by dividing the number of subjects in the control group by the number of comparisons while retaining the mean and SD of the change according to the Cochrane Handbook (40).

The changes in 25(OH)D concentrations were calculated as weighted mean differences with 95% CIs. Statistical heterogeneity between the studies was tested by using the Cochrane Q-test (41). A random-effects model was applied. Publication bias was assessed by a funnel plot (**Figure 1**) (42). In addition to the main analysis, we conducted several sensitivity analyses taking into account study duration, type of fish, mean baseline 25(OH)D concentrations, season of blood collection, access to individual data or calculated data, participants' health status, the amount of fish consumed during the trial, measurements of total 25(OH)D or 25-hydroxycholecalciferol [25(OH)D_3], the determination of the vitamin D content in the fish, and the method of determination of 25(OH)D concentrations [ELISA/radioimmunoassay or liquid chromatography–tandem mass spectrometry (LC-MS/MS)]. Two studies (27; U Lehmann, unpublished data, 2012) compared fish with different vitamin D contents. These 2 fish interventions were compared in an additional separate analysis.

Included studies

In addition to published studies, we included 2 unpublished RCTs involving fish consumption in healthy adults. One of these was conducted at the University of Bergen in Norway and the

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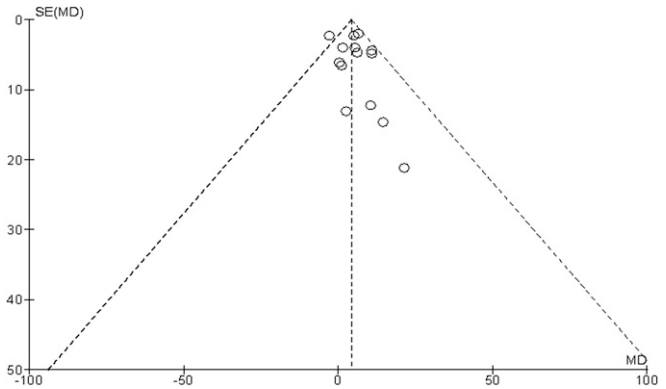

FIGURE 1 Funnel plot with pseudo 95% CIs for the effect of fish intake on serum 25-hydroxyvitamin D concentrations. MD, mean difference.

other at the Martin Luther University of Halle-Wittenberg in Germany. These studies are described briefly below and in greater detail in the **Supplemental Text**.

Lehmann study

The study in Halle (Saale) at the Martin Luther University of Halle-Wittenberg (latitude 51° north) was conducted during the late autumn of 2012. The major aim was to compare the effect of vitamin D–enriched rainbow trout on 25(OH)D_3 concentrations in comparison with conventional rainbow trout in healthy volunteers (n = 53) over a 4-wk period. The participants consumed 6 times/wk 100-g portions of rainbow trout enriched with vitamin D by postmortem irradiation with UV-B or 100-g portions of conventional, untreated rainbow trout fillets. Consumption was usually at lunchtime and was supervised on weekdays. Both participants and investigators were blinded to the type of trout. Blood samples were collected at baseline and after 4 wk for determination of 25(OH)D_3 concentrations by LC-MS/MS (MassChrom 25-OH Vitamin D_3 reagent kit for LC-MS/MS analysis; Chromsystems GmbH) on an API2000 LC-MS/MS system (Applied Biosystems), as described elsewhere (51). Characteristics of participants are provided in **Supplemental Table 2**.

Gudbrandsen study

This was a randomized controlled intervention study with a parallel design and 3 intervention arms: cod, salmon, or chicken in weekly doses of 750 g (5 meals of 150 g)/wk for 4 wk, with study visits at baseline and after 4 wk. The study included 57 participants recruited in Bergen, Norway, and randomly assigned to the intervention groups. Because of the reduced number of blood samples (n = 5 with missing data) and dropouts (n = 3), samples for the 25(OH)D analyses were only available for 19, 18, and 12 participants, respectively. Fasting blood samples were collected at baseline and after 4 wk, and 25(OH)D was determined in serum by LC-MS/MS according to methods of Midttun and Ueland (45). Characteristics of participants are provided in **Supplemental Table 3**.

RESULTS

A systematic search of the literature led to the identification of 3277 possibly relevant articles (**Figure 2**). A first examination identified 61 studies as appropriate to be included in the analysis by reviewing titles and abstracts. After detailed consideration, 54 studies were rejected from the analysis, because they did not measure 25(OH)D as an outcome, were not RCTs, gave no detailed information on amount of fish, or were duplicates of included studies. In total, 7 published and 2 unpublished studies that fulfilled the inclusion criteria were included in the present meta-analysis investigating the effect of fish intake on serum 25(OH)D concentrations.

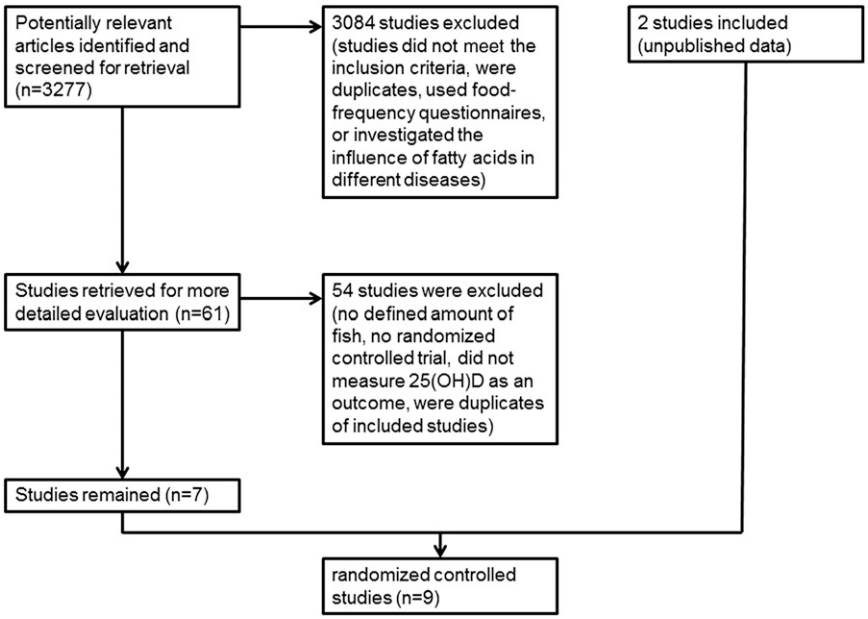

FIGURE 2 Flow diagram for the selection of studies of the effects of fish intake on serum 25(OH)D concentrations in the present meta-analysis, indicating numbers of articles reviewed and later excluded or included for the meta-analysis. 25(OH)D, 25-hydroxyvitamin D.

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TABLE 1
Characteristics of the randomized controlled trials included in the present meta-analysis[1]

Authors (ref)	Year	Country	Intervention, dose, and frequency[2]	Included in analysis, *n*	Sex and age	Follow-up	Baseline 25(OH)D (fish group), nmol/L
Erkkilä et al. (26)[3]	2008	Finland	*1*) Control (lean meat or chicken) [10]	33	27 men, 6 women; 61.0 ± 5.8 y	8 wk	—
			2) Lean fish (400–600 g/wk) [11]				96 ± 30[4]
			3) Fatty fish (400–600 g/wk) [12]				124 ± 43
Lucey et al. (37)[3]	2008	Iceland, Ireland, Spain	*1*) Control (sunflower oil, 3 g/d) [66]	210	92 men, 118 women; 20–40 y	8 wk	—
			2) Cod (450 g/wk) [70]				59.0 ± 22.1
			3) Salmon (450 g/wk) [74]				61.9 ± 33.8
Pot et al. (28)[3]	2009	United Kingdom, Netherlands	*1*) Control (dietary advice) [23]	74	37 men, 37 women; 18–79 y	6 mo	—
			2) Cod (300 g/wk) [22]				71.6 ± 30
			3) Salmon (300 g/wk) [29]				71.7 ± 26.5
Hallund et al. (27)[3]	2010	Denmark	*1*) Control (chicken, 1050 g/wk) [22]	68	All men; 40–70 y	8 wk	—
			2) Trout raised on marine feed (1050 g/wk) [23]				45.9 ± 20.9
			3) Trout raised on vegetable-based feed (1050 g/wk) [23]				48.1 ± 22.1
Hansen et al. (34)	2010	Norway	*1*) Control (alternative dinner) [14]	29	All men; 20–60 y	23 wk	
			2) Seafood, mainly salmon (600 g/wk) [15]				48 ± 15
OA Gudbrandsen (unpublished data, 2014)[3]	2013	Norway	*1*) Chicken (750 g/wk) [12]	49	16 men, 33 women; 20–36 y	4 wk	—
			2) Cod (750 g/wk) [18]				66.5 ± 17.8
			3) Salmon (750 g/wk) [19]				77.0 ± 23.1
U Lehmann (unpublished data, 2012)	2013	Germany	*1*) Control (common rainbow trout) (600 g/wk) [27]	53	24 men, 29 women; 20–63 y	4 wk	
			2) Vitamin D–enriched rainbow trout (600 g/wk) [26]				44.8 ± 14
Scheers et al. (35)	2013	Sweden	*1*) Control (650 g pork or 750 g chicken/wk) [21]	21	All men; 35–60 y	2 × 6 wk (crossover)	
			2) Herring (750 g/wk) [21]				66.9 ± 22.1
Hansen et al. (25)	2014	Norway	*1*) Control (3 times meat/wk) [42]	82	All men; 18–61 y	6 mo	
			2) Salmon (900 g/wk over 5 mo, 450 g/wk during the past 4 wk) [40]				85 ± 36

[1] ref, reference; 25(OH)D, 25-hydroxyvitamin D.
[2] *n* in brackets.
[3] These studies included 2 intervention groups (fatty compared with lean fish).
[4] Mean ± SD (all such values).

TABLE 2
Quality evaluation of the 9 randomized controlled studies included in the meta-analysis[1]

Authors, year (ref)	Compliance	Dropouts, *n* (%)	Measured vitamin D content in fish	Intervention period	Analytic method for 25(OH)D	Randomization	Participants	Quality score[2]
Erkkilä et al., 2008 (26)	Checked by self-report and serum fatty acid composition	2 (5.7)	No data	Spring, August–September, October	ELISA	Stratified by sex	Survivors of myocardial infarction	4
Lucey et al., 2008 (37)	86%	34 (13.9)	Data cited but not measured (9.6 μg/100 g)	October–May	ELISA	No information	Overweight; consuming a low-calorie diet	4
Pot et al., 2009 (28)	Serum n–3 very-long-chain PUFAs	22 missing blood samples	No information	November 2004–July 2007	ELISA	Randomization in blocks (n = 6)	Only participants with healthy colon included	5
Hallund et al., 2010 (27)	99% evaluated in study diaries	7 (9.3)	Measured trout raised on marine feed: 0.62 μg/100 g; trout raised on vegetarian feed: <0.1 μg/100 g	Spring or autumn	Chemiluminescence immunoassay	Randomization without taking baseline characteristics into account	Healthy volunteers	6
Hansen et al., 2010 (34)	No information	24 (45.3)	No data	April–November	Radioimmunoassay	No information	Prisoners	3
OA Gudbrandsen (unpublished data, 2014)	Checked by self-report	8 (14)	No data	Winter	LC-MS/MS	Consideration of sex, BMI, and age	Healthy volunteers	4
U Lehmann (unpublished data, 2012)	93%	4 (7)	2.8 μg/100 g in intervention trout; 0.6 μg/100 g in control trout	November–December	LC-MS/MS	Randomization in blocks (n = 2); consideration of sex, BMI, and 25(OH)D_3 concentrations at screening	Healthy volunteers	6
Scheers et al., 2013 (35)	Checked by self-report (24-h recall) and fatty acid concentration in blood	n = 5 dropouts; n = 19 for this analysis who were missing blood samples for 25(OH)D (19.2)	8.5 μg/100 g vitamin D in herring	April–June, September–November	HPLC	No randomization because crossover study	Healthy, overweight	6
Hansen et al., 2014 (25)	No information	11 (11.8)	15 μg/300 g in salmon but no source added	September – February	Chemiluminescence immunoassay	No information	Sex offenders	3

[1]The quality score was based on assessment of compliance, number of dropouts, measurements of vitamin D content in fish, season of intervention, type of vitamin D analysis, and type of randomization. One score point was given for each item of information included. LC-MS/MS, liquid chromatography–tandem mass spectrometry; ref, reference; 25(OH)D, 25-hydroxyvitamin D; 25(OH)D_3, 25-hydroxycholecalciferol.

[2]Scores of study quality: 5–6 denotes good quality, 3–4 indicates moderate quality, and 0–2 points denotes low quality.

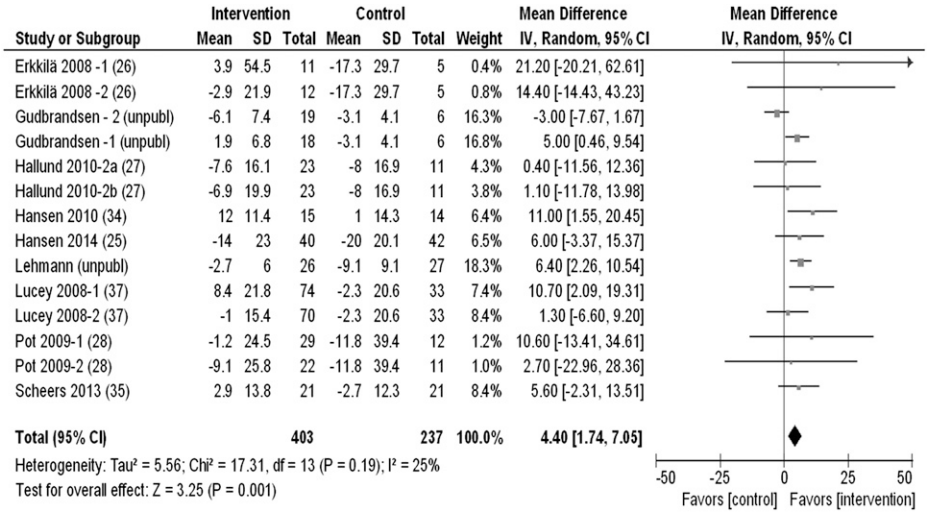

FIGURE 3 Random-effects meta-analysis comparing the effects of fish intervention with the control food on the 25(OH)D concentrations (in nmol/L). The forest plot indicates a significant effect of the absolute change in 25(OH)D concentrations compared with the control food by fish intervention. "Total" denotes the cumulative *n* from all of the included studies. IV, inverse variance; unpubl, unpublished; 25(OH)D, 25-hydroxyvitamin D; −1, study groups who received fatty fish; −2, study groups who received lean fish.

Study characteristics

The 9 studies considered comprised 619 participants [640 participants were included in the meta-analysis on account of 1 crossover study (34)] aged between 18 and 79 y. Descriptive study information is shown in **Table 1**. Sixty-four percent of the study population were men (n = 396) and 36% were women (n = 223). The studies were conducted in Finland, Iceland, Ireland, Spain, the United Kingdom, Netherlands, Denmark, Norway, the United States, Germany, and Sweden. Two studies (28, 37) were multicenter studies. Most of the participants were white, although a number of studies did not specify this explicitly. The change in 25(OH)D concentration served as the primary outcome in only 1 case (U Lehmann, unpublished data, 2012), whereas in the other studies the 25(OH)D concentration was measured as a secondary outcome (25–28, 34, 35) or was measured post hoc (OA Gudbrandsen, unpublished data, 2014).

The interventions differed between the studies in dosage, time, and fish species. We included 3 long-term studies with an intervention period of 6 mo or 23 wk (25, 28, 34). Six studies (26, 27, 35, 37; OA Gudbrandsen, unpublished data, 2014; U Lehmann, unpublished data, 2012) investigated the influence of short-term fish intake (4–8 wk) on 25(OH)D concentrations. The planned amount of fish to be consumed varied from 300 to 1050 g/wk. In 6 studies the weekly fish intake was planned to be between 300 and 600 g (25, 26, 28, 34, 37; U Lehmann, unpublished data, 2012), whereas in 3 studies (27, 35; OA Gudbrandsen, unpublished data, 2014) the intake was between 750 and 1050 g. The selected fish species differed between studies. The intake of fatty fish (salmon, herring) was investigated in 3 studies (25, 34, 35), whereas 4 studies compared both fatty and lean fish (cod, rainbow trout; 26, 28, 37; OA Gudbrandsen, unpublished data, 2014). One study included rainbow trout in the fatty fish group (26), and 2 studies investigated rainbow trout that differed in either the feeding regimen (27) or in postmortem treatment (U Lehmann, unpublished data, 2012). Total serum 25(OH)D concentrations measured by ELISA/immunoassay were reported in 6 studies (25–28, 34, 37), whereas in 3 studies serum 25(OH)D_3 concentrations were measured by chromatographic methods (35; U Lehmann, unpublished data, 2012; OA Gudbrandsen, unpublished data, 2014).

All of the studies were designed as RCTs and included a control group. Because of the visible differences between the meals, 8 studies were not blinded. Only 1 study (U Lehmann, unpublished data, 2012) was double-blinded. Details of the randomization scheme and criteria were reported in 4 cases (27, 28; OA Gudbrandsen, unpublished data, 2014; U Lehmann, unpublished data, 2012), and 1 study had a crossover design (35). All of the studies provided general information on season of the intervention period, but an exact timing (month or season) of the blood collection procedures was usually not possible. Exact compliance rates were reported in only 3 studies (27, 37; U Lehmann, unpublished data, 2012), but the drop-out rates were given in all studies (25–28, 34, 35, 37; OA Gudbrandsen, unpublished data, 2014; U Lehmann, unpublished data, 2012).

Individual data were available for 6 of the 9 studies [298 participants, although 319 individual data are included because of 1 crossover study (35)] to calculate the change in 25(OH)D concentrations (26–28, 35; OA Gudbrandsen, unpublished data, 2014; U Lehmann, unpublished data, 2012), whereas the mean (SD) 25(OH)D concentrations at baseline and during follow-up in the study groups were available in 3 studies (25, 34, 37). The vitamin D_3 concentration in the fish was reported in 3 studies (27, 35; U Lehmann, unpublished results) but was explicitly measured only by Hallund et al. (27) and Lehmann (unpublished data, 2012). Average fish vitamin D_3 concentrations were cited by 2 studies (25, 37).

Results of the study quality assessment are presented in **Table 2**. The quality score was high in 5 studies (27, 28, 35; OA Gudbrandsen, unpublished data, 2014; U Lehmann, unpublished data, 2012) and moderate in 4 studies (25, 26, 34, 37). None of the studies had a low score.

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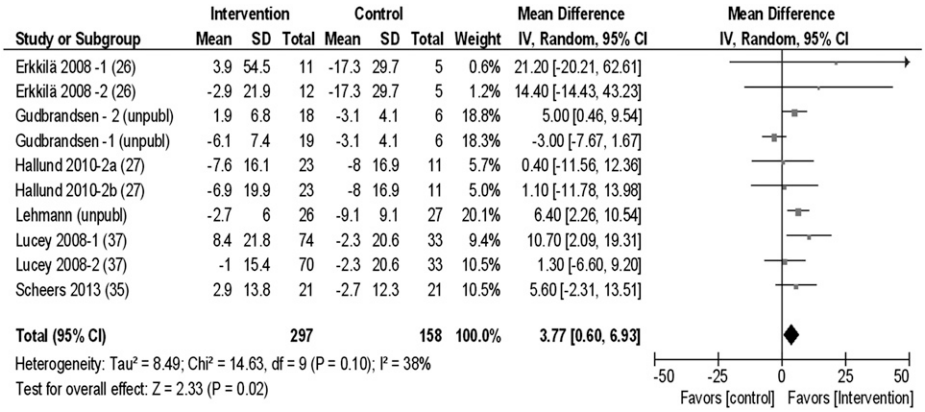

FIGURE 4 Random-effects meta-analysis comparing the effects of short-term (4–8 wk; 10 study groups) fish intervention with the control food on 25(OH)D concentrations (in nmol/L). The forest plot indicates a significant effect of the absolute change in 25(OH)D concentrations compared with the control food by fish intervention. "Total" denotes the cumulative *n* from all of the included studies. IV, inverse variance; unpubl, unpublished; 25(OH)D, 25-hydroxyvitamin D; −1, study groups who received fatty fish; −2, study groups who received lean fish.

Meta-analysis

Comparison of the changes in 25(OH)D concentrations between the fish intervention and the control groups including all studies (n = 9; 14 study groups) resulted in a weighted mean difference of 4.4 nmol/L (95% CI: 1.7, 7.1 nmol/L; $P < 0.001$, $I^2 = 25\%$) (**Figure 3**).

The effect of the fish intervention varied depending on the study duration. Short-term studies (4–8 wk) showed a mean difference of 3.8 nmol/L (95% CI: 0.6, 6.9 nmol/L; $P < 0.02$, $I^2 = 38\%$), whereas long-term studies (∼6 mo) showed a mean difference of 8.3 nmol/L (95% CI: 2.1, 14.5 nmol/L; $P < 0.009$, $I^2 = 0\%$) (**Figures 4** and **5**).

The type of fish also had an effect. Fatty fish (salmon, herring; n = 7 study groups) resulted in a mean difference of 6.8 nmol/L (95% CI: 3.7, 9.9 nmol/L; $P < 0.0001$, $I^2 = 0\%$), whereas studies that used lean fish (trout, cod; n = 6 study groups) showed a mean difference of −1.1 nmol/L (95% CI: −4.7, 2.5 nmol/L; $P < 0.55$, $I^2 = 0\%$). When the unpublished study by Lehmann was included (n = 7 study groups), which used lean fish that were biofortified with vitamin D, the weighted mean difference changed to 1.9 nmol/L (95% CI: −2.3, 6.0 nmol/L; $P < 0.38$, $I^2 = 37\%$) (**Figures 6** and **7**).

In 2 studies, different types of rainbow trout were compared: the intervention group received trout that had been biofortified either by the feeding regimen (26) or by postmortem irradiation (U Lehmann, unpublished data, 2012). A separate meta-analysis of these studies showed a weighted mean difference of 5.4 nmol/L (95% CI: 1.6, 9.1 nmol/L; $P < 0.005$, $I^2 = 0\%$) between the intervention groups and controls.

An additional sensitivity analysis was carried out to investigate the influence of the mean baseline 25(OH)D concentration. In 3 studies that included 4 study groups, mean baseline 25(OH)D in the intervention groups was <50 nmol/L (27, 34; U Lehmann, unpublished data, 2012). The weighted mean difference was 6.1 nmol/L (95% CI: 2.7, 9.6 nmol/L; $P < 0.0006$, $I^2 = 0\%$), compared with 3.9 nmol/L (95% CI: 0.4, 7.3 nmol/L; $P < 0.03$, $I^2 = 30\%$) in 6 studies with 10 study groups in which mean baseline 25(OH)D concentrations were >50 nmol/L. A meta-analysis that used individual patients' data that were available from 6 trials (26–28, 35; OA Gudbrandsen, unpublished data, 2014; U Lehmann, unpublished data, 2012) did not show results that were different from those in the analysis of aggregated data (data not shown).

DISCUSSION

In this meta-analysis we investigated whether fish intake increases serum 25(OH)D concentrations in healthy adults under controlled conditions and included 9 RCTs with good or moderate quality. The main result was that the consumption of at least 2 fish meals, corresponding to ∼300 g fish/wk over a period of at least 4 wk, was associated with a significant increase in 25(OH)D

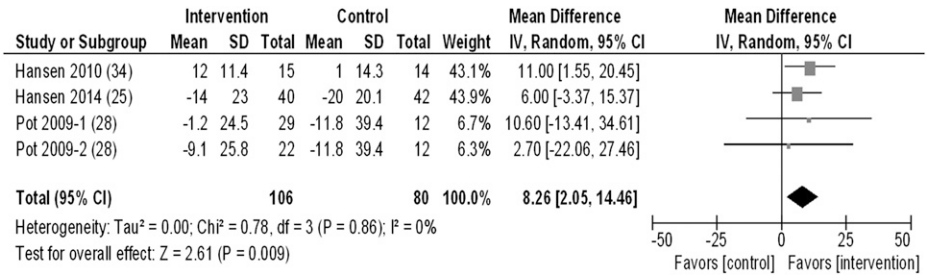

FIGURE 5 Random-effects meta-analysis comparing the effects of long-term (6 mo; 4 study groups) fish intervention with the control food on 25(OH)D concentrations (in nmol/L). The forest plot indicates a significant effect of the absolute change in 25(OH)D concentrations compared with the control food by fish intervention. "Total" denotes the cumulative *n* from all of the included studies. IV, inverse variance; 25(OH)D, 25-hydroxyvitamin D; −1, study groups who received fatty fish; −2, study groups who received lean fish.

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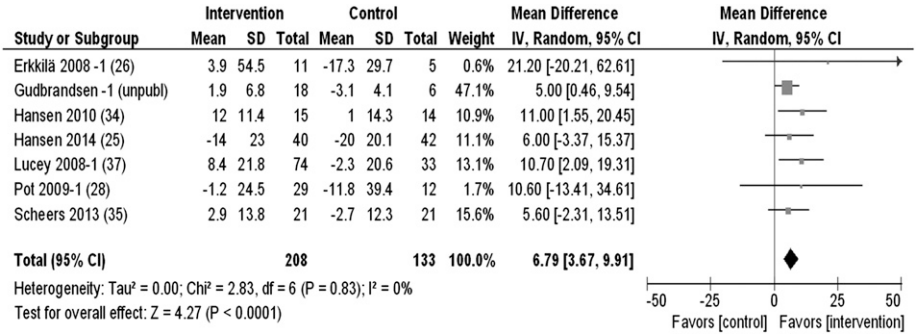

FIGURE 6 Random-effects meta-analysis comparing the effects of fatty fish intervention (7 study groups) with the control food on 25(OH)D concentrations (in nmol/L). The forest plot indicates a significant effect of the absolute change in 25(OH)D concentrations compared with the control food by fish intervention. "Total" denotes the cumulative *n* from all of the included studies. IV, inverse variance; unpubl, unpublished; 25(OH)D, 25-hydroxyvitamin D; −1, study groups who received fatty fish.

concentrations. In addition, fatty fish, longer study durations, and lower baseline 25(OH)D concentrations were associated with larger increases in 25(OH)D concentrations.

Although it has long been known that the consumption of fish is healthy, and this finding is included in most dietary recommendations, so far there have been few quantitative analyses supporting this effect on intermediate endpoints. The present analysis adds to our knowledge of the health-related effects of fish consumption and allows a quantitative estimate of what may be expected from increased fish consumption. This type of analysis is not very common in nutrition research and has not so far been included in recommendations and guidelines.

The present meta-analysis showed that the type of the fish is an important factor. Lean fish, mostly cod, did not increase vitamin D status to a significant extent, although it should be noted that differentiation between lean and fatty may be arbitrary in some species that could also be classified as medium-fatty fish. We observed a significant increase in 25(OH)D concentrations only when biofortified rainbow trout was included in the lean fish group. Thus, the consumption of fatty or biofortified fish should be recommended from the standpoint of improving vitamin D status.

In 2 studies, different types of rainbow trout were investigated: fish that were biofortified with vitamin D either by feeding or by postmortem exposure to UV-B radiation. These studies showed that there is a huge potential for improving the vitamin D content, which is more pronounced by using postmortem irradiation than by feeding. However, both technologies should be developed further, because the absolute amounts of vitamin D in the treated fish were still relatively low. In this respect, it is interesting to note that preliminary data on freshwater fish species also indicate an effect of different living conditions on vitamin D content (46).

One side effect of high fish consumption may be an increased exposure to environmental toxins that accumulate in fish and in seafood. Health authorities such as the Norwegian Scientific Committee for Food Safety therefore recommended in 2007 an upper intake limit of 400 g fatty fish/wk (47). It has been shown that the accumulation of toxins was high in fatty fish species such as herring, salmon, and sprat (48). Within the same fish species contamination may vary depending on age, fat content, and geographic region (49). In our meta-analysis none of the studies investigated the association between fish intake and toxins, but this clearly should be taken into account when recommending high fatty fish intakes to improve vitamin D status and should be explored in future studies.

Our knowledge of the variation in vitamin D content in fish is limited. For example, the vitamin D content of the fish used throughout the study was only measured in 2 studies (27; U Lehmann, unpublished data, 2012). It may be assumed that, even within a given fish species, there is a wide variation in vitamin D content depending on growth, feed, and other factors such as season (13). For example, it has been hypothesized that farmed

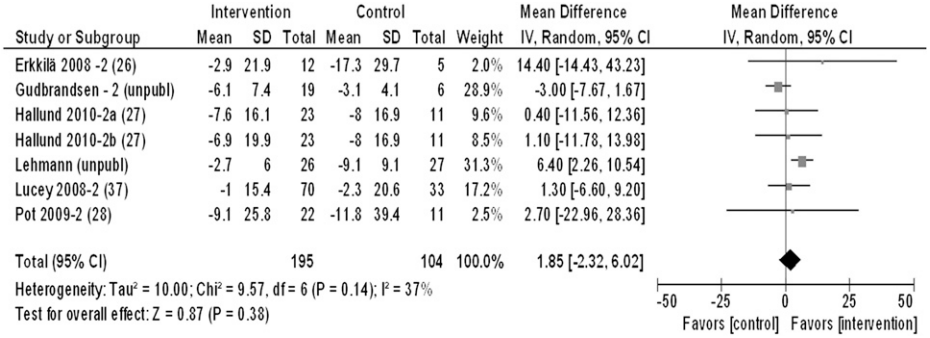

FIGURE 7 Random-effects meta-analysis comparing the effects of lean fish intervention (7 groups) with the control food on 25(OH)D concentrations (in nmol/L). The forest plot indicates a nonsignificant effect of the absolute change in 25(OH)D concentrations compared with the control food by fish intervention. "Total" denotes the cumulative *n* from all of the included studies. IV, inverse variance; unpubl, unpublished; 25(OH)D, 25-hydroxyvitamin D; −2, study groups who received lean fish.

salmon contains less vitamin D than does wild salmon (13, 50). Thus, there is a need for more detailed and accurate data on the determinants of vitamin D content in wild and farmed fish.

Although fish is one of the few foods that contain vitamin D (12), there is still an ongoing discussion whether fish intake contributes to a sufficient supply of vitamin D. Several observational studies (51–53) investigated the relation of fish intake and vitamin D status with the aid of food-frequency questionnaires. With the use of data from EPIC (European Prospective Investigation into Cancer and Nutrition)–Germany, Kühn et al. (51) reported a low, but positive, significant association between fish intake and 25(OH)D concentrations. In the United Kingdom, people who consumed meat and fish had higher 25(OH)D concentrations than did vegetarians and vegans (52). In Swedish women, fatty fish was one important predictor of serum 25(OH)D concentrations (53).

On the basis of all of the available data on fish intake, we observed a mean increase of 4.4 nmol 25(OH)D/L and an increase of 6.8 nmol/L when only fatty fish was considered. The application of the proposed increase of 25(OH)D of 1.97 nmol/L per additional microgram of vitamin D intake (54) suggests an intake of 2.2 μg vitamin D/d for all types of fish and 3.5 μg vitamin D/d for fatty fish such as salmon. According to the available data from food-composition tables, 300 g raw salmon/wk (corresponding to 2–3 portions) would provide 4.3, 6.9, or 2.5 μg vitamin D/d when using Norwegian, German, or UK databases, respectively (55–57). Whether these differences reflect natural variation or differences in analytic methods is unclear at present. Efforts to harmonize food-composition databases have been undertaken, e.g., by European Food Safety Authority or the EuroFIR project (www.eurofir.org).

The above calculations also show clearly that this fish intake is insufficient and does not fulfill the revised recommendations for a daily dietary amount of vitamin D that would improve vitamin D status (58–61). Indeed, it may be misleading to recommend fish consumption alone to improve vitamin D status. A daily intake of 2.2 or 3.5 μg vitamin D—which is achieved by consuming $\sim$300–600 g fish/wk—will not increase serum 25(OH)D concentrations to an optimal level (>50 nmol/L) in most people and will only result in increases of 4.4 or 6.8 nmol/L, respectively. Our results are in line with dose-response studies conducted in older adults (54), which showed that subjects supplemented with 5 μg vitamin D/d were able to maintain 25(OH)D concentrations during wintertime, whereas supplementation with 10 μg/d increased 25(OH)D concentrations by $\sim$12 nmol/L, on average. In healthy postmenopausal women, a daily supplement of 800 IU vitamin D (corresponding to 20 μg) was needed to increase 25(OH)D concentrations to >50 nmol/L in almost all of the women (62).

Strengths and limitations of the study

A major strength of the study is the inclusion of only RCTs and the collection of individual patients' data for at least some of the studies. All of the studies reached either a high- or moderate-quality score, although the use of established quality scores was prevented by the use of real food and therefore lack of participant blinding in 8 of the 9 studies. Limitations included that, due to the low number of studies, no further sensitivity analyses with respect to the amount or type of fish, length of intervention, or analytic method for determination of 25(OH)D were possible. In particular, the different analytic methods used for 25(OH)D measurements may have affected the results, because only 3 studies used chromatographic (LC-MS/MS or HPLC) methods.

Conclusions

We conclude that fish, as an important food source of vitamin D, increases 25(OH)D concentrations but cannot optimize vitamin D status. The side effects of the accumulation of environmental pollutants must be taken into account and investigated further. It should be clarified which foods are effective in improving vitamin D status; however, it seems to be difficult to increase the vitamin D concentrations sufficiently without using either supplements or fortified food in the absence of UV-B radiation.

The authors' responsibilities were as follows—UL and JD: designed the study and applied for funding; UL, HRG, and JD: performed the literature search and the meta-analysis; FH, AM-B, OAG, PMU, GM, GIS, and JD: were involved in the planning, design, and conduct of the 2 unpublished studies; LL, HL, ALH, ATE, and GKP: provided original data from their studies; and all authors: were substantially involved in the writing process. None of the authors stated a conflict of interest.

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