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RESEARCH ARTICLE

Fish liver and seagull eggs, vitamin D-rich foods with a shadow: Results from the Norwegian Fish and Game Study

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Scope: Fish liver, fish liver oil, oily fish and seagull eggs have been major sources of vitamin D for the coastal population of Norway. They also provide dioxin and polychlorinated dioxin-like compounds (dl-compounds), which may interfere with vitamin D homeostasis. We investigated whether serum 25-hydroxyvitamin D (25(OH)D) might be compromised by concomitant intake of dl-compounds.

Methods and results: We studied 182 adults participating in the Norwegian Fish and Game Study. Participants who consumed fish liver and/or seagull eggs had higher dl-compound intake and blood concentrations than non-consumers ($p < 0.001$). Vitamin D intake was higher ($p < 0.001$), whereas serum 25(OH)D was lower ($p = 0.029$) in consumers than in non-consumers. Among non-consumers, vitamin D intake was associated with serum 25(OH)D ($\beta = 1.06$; 95% CI: 0.48, 1.63). This association was weaker among consumers ($\beta = 0.52$; 95% CI: −0.05, 1.08), but strengthened when adjusted for retinol intake ($\beta = 0.66$; 95% CI: 0.12, 1.21). The association between vitamin D intake and serum 25(OH)D did not seem to be compromised by intake of dl-compounds.

Conclusion: To secure adequate vitamin D status while keeping the intake of dioxins and dl-polychlorinated biphenyls low, a healthy diet should include both supplemental vitamin D and oily fish. Despite high nutrient content, dietary fish liver and seagull eggs should be restricted, due to dl-compounds and possible vitamin A-D antagonism.

Keywords:
Fish / Fish oil / 25-Hydroxy-vitamin D / Total toxic equivalent / Vitamin D

1 Introduction

Fish is a well-known source of nutrients like high-quality protein, iodine, selenium, vitamin D and long-chain n-3 fatty acids. However, fish is also a source of contaminants, such as persistent organic pollutants (POPs). The diversity in contents of nutrients and contaminants might partly explain the discrepancy between studies investigating the association between fish consumption and health [1–4]. Accordingly, national and international recommendations to eat more fish need to be balanced with concerns about pollutants [1, 3, 5]. Globally, solar ultraviolet (UV) radiation is the main contributor to vitamin D status through the cutaneous photoconversion of pro-vitamin D to pre-vitamin D. However, in countries located in the far north, like Norway, sun-induced vitamin D production ceases during the winter months, and the population becomes entirely dependent on food sources for its vitamin D supply. Oily fish is one of the few natural food sources of vitamin D. In addition, the liver of lean fish is a rich source of the vitamin and has traditionally been part

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Abbreviations: dl-compounds, dioxin-like compounds; dioxin and dioxin-like PCBs; **PCBs**, polychlorinated biphenyls; **POPs**, persistent organic pollutants; **TEF**, toxic equivalency factor; **TEQ**, toxic equivalents; **25(OH)D**, 25-hydroxy-vitamin D; **TWI**, tolerable weekly intake

of the diet of many coastal communities. Seagull eggs (*Larus* species) have also provided ample vitamin D in the late spring season when a number of coastal communities have had a tradition for collection and consumption. Inland communities with little or no access to fish, by contrast, used to have a much higher incidence of rickets. For the last 60 years, the Norwegian health authorities have recommended a small spoon of cod-liver oil ($\approx$ 5 mL) daily throughout the winter months to ensure an adequate intake of vitamin D (up to 10.0 μg/day) [6]. The last decade, all butter and margarine brands, and one brand of milk, have been fortified with vitamin D.

In addition to its well-known function in bone metabolism, vitamin D has recently been linked to the prevention of different diseases [7–11]. While vitamin D has been associated with lower risk of diabetes [7], POPs have been found to increase the risk for insulin resistance syndrome [12]. In the wake of the broadened understanding of the physiological role of vitamin D in the body, discussions are now focusing on whether the recommended Nordic dietary intake (7.5 μg/day if <60 years and 10 μg/day if >60 years) [13] should be increased [14]. However, given the polychlorinated dioxin and furans (dioxins) and polychlorinated biphenyls (PCBs) concentrations found in fish and other seafood, the best strategy for securing a sufficient supply of vitamin D and other nutrients in the daily diet, while at the same time avoiding excessive intakes of contaminants, is still an open question. Furthermore, interference of contaminants on steroid and xenobiotica receptor (SXR) activation and a subsequent effect on vitamin D homeostasis is possible [15, 16].

Persistent exposure to organic pollutants such as dioxins and PCBs is considered detrimental to human health [12, 17–19] and a level for tolerable weekly intake (TWI) of dioxins and dioxin-like PCBs (dl-PCBs) (dl-compounds) was established by the EU Scientific Committee on Food in 2001 [20]. Since these compounds tend to accumulate in seafood, the Norwegian Scientific Committee for Food Safety (VKM) carried out a comprehensive assessment of fish and other seafood in 2007. The Committee proposed an upper intake level of two oily fish meals per week (400 g). This proposal was based on dietary model estimates and the average food levels of dioxins and dl-PCBs (dl-compounds) at the time (The toxicities of the different congeners of dioxins and dl-PCBs have been ranked relative to 2,3,7,8-TCDD. This is the most potent dioxin and has been assigned a toxic equivalency factor (TEF) of 1. Less toxic congeners have a TEF of <1, e.g. 0.001 [21, 22]. The TEF values allow the total amount of all the dl-compounds, i.e. the sum of PCDDs/PCDFs and dl-PCBs, to be expressed as 2,3,7,8-TCDD toxic equivalents (TEQs). TEQ is obtained by multiplying the concentration of each congener by its TEF, and total TEQ = sum of TEQ from individual congeners). Consumption of highly contaminated food items such as seagull eggs and fish liver was discouraged due to high content of dl-compounds. If followed, this diet would ensure that the TWI would not be exceeded [23] (http://www.vkm.no/dav/d94dff429b.pdf), while at the same time meeting the need for nutrients like vitamin D and essential fatty acids.

The Norwegian Fish and Game (NFG) Study was designed to investigate levels of exposure to food-derived contaminants in Norway. Adults from 27 municipalities in which fishing and hunting is commonplace, and who reported frequent intake of food known to be high in different contaminants such as dioxins and PCBs, were invited to participate, as was a smaller random sample of the general population of these municipalities. As some of these foods are also important sources of fat-soluble vitamins, the unique composition of the study group, combined with the extensive dietary data and blood samples collected, provided a rare opportunity to investigate the real-life association between dietary vitamin D intake and dl-compound exposure and the blood status of these components. The aim of the present study was to investigate whether the intake of vitamin D-rich foods resulted in unacceptably high intake of dl-compounds and whether intake of dl-compounds affected the association between vitamin D intake and concentration of 25-hydroxyvitamin-D (25(OH)D).

2 Materials and methods

2.1 NFG Study

We used data from participants of the Part C of the NFG Study, whose objective was to describe the range of dietary exposure to environmental contaminants in the Norwegian population. Details of the study have been published previously [24, 25]. In part B of the NFG study, participants were recruited from coastal and inland municipalities in Norway with ample supplies of fish and/or game. This part of the study included 5500 participants (age, 18–79 years), each of whom answered a 4-page semi-quantitative food frequency questionnaire, mainly asking about consumption of fish and game [26]. From these answers, an estimate of the individual intakes of PCBs, dioxins, mercury and cadmium was established based on the content of different contaminants in the food items [25]. Participants for part C of the NFG Study were recruited from this pool, using a two-step inclusion strategy. Participants with an estimated high intake of PCBs, dioxins, mercury and cadmium (n = 434) were invited first. We then randomly selected and invited participants from the remaining population to form a reference group (n = 267). This was done to secure both enough participants with a presumed high intake of the contaminants in question as well as participants covering a wide range of intakes. In contrast to many other epidemiological studies, the intention in part C was not to obtain a representative sample of the population, but to include consumers with a wide distribution of intake and to identify sub-groups of the population which may be assumed to have a substantial consumption of foods high in contaminants. The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate (id: S-02138).

2.2 Response rate

The response rate was close to 28% for both groups, resulting in 194 participants, 117 in the group with a high estimated intake of contaminants and 77 in the randomly selected reference group. No significant differences were found with regard to demographic factors or the estimated contaminant exposure from diet except that participants were slightly older and included a higher percentage of university graduates than the non-participants (results not shown).

2.3 Implementation

After being contacted by mail and giving informed consent in accordance with the Helsinki Declaration, participants answered a semi-quantitative food frequency questionnaire (FFQ), covering their habitual diet during the last 12 months and a 1-page demographic questionnaire. Each of the participants had to make an appointment with their physician to have a blood sample taken, at which time they also delivered a morning urine sample.

2.4 Food frequency questionnaire

The food frequency questionnaire was adapted to Norwegian food traditions, including questions on consumption of fish liver and seagull eggs, and was originally developed for the Norwegian Mother and Child Cohort Study (MoBa) [27]. The 12-page questionnaire has been thoroughly described and validated in a pregnancy sub-cohort [28], and was found to be a valid tool for measuring habitual intake and for ranking pregnant women according to high and low intakes of energy, nutrients and food. In the present study, ten participants were excluded from the analysis due to unlikely energy intakes (<1000 or more than 4000 kcal/day), and two due to missing data on the serum concentration of 25(OH)D, resulting in a total of 182 participants. The questionnaires were optically read. FoodCalc [29] (http://www.ibt.ku.dk/jesper/foodcalc (Accessed February 2006)) and the Norwegian food composition table [30] (www.matportalen.no/Matvaretabellen (Accessed 2003)) were used to calculate the nutrient and contaminant values of foods and dishes. The vitamin D and retinol concentrations of seagull eggs were analysed for the purpose of this study (Supporting Information A).

2.5 Databases

A database of the declared content of dietary supplements was developed for the calculation of nutrient intake from supplements. Cholecalciferol (vitamin D3) is the predominant form of vitamin D found in supplements containing vitamin D sold in Norway [31]. In this study, we made use of an extensive database containing records of all available concentrations of dioxins and PCB congeners in Norwegian foods from 2000 to 2006 [25]. In this study, we have primarily addressed the exposure to dioxins and dioxin-like PCBs, expressed in terms of total TEQs intake. There is, however, a strong correlation between the dietary intake of the dl-compounds and the non-dl-PCBs (ndl-PCBs) among Norwegians [25]. More details about the database are described elsewhere [25].

2.6 Methods of analysis – 25(OH)D, retinol and dl-compounds in serum and blood

Serum vitamin D ($25(OH)D_2$ and $25(OH)D_3$) and retinol were determined by means of liquid chromatography–mass spectrometry (LC-MS) [32]. PCDD/PCDF and dl-PCB analyses were carried out on whole blood samples from participants who had already had their serum ndl-PCB concentrations measured as described previously [25]. Lipids in blood were determined enzymatically, and the total lipid content of the samples was calculated in accordance with a previously described method [33].

2.7 Statistical methods

While the serum 25(OH)D concentration was normally distributed, the dietary intakes of seafood, nutrients and toxicants were not all normally distributed. Data are therefore presented as percentages or medians, 5th and 95th percentiles. Differences between groups were examined using the χ^2-square test for nominal variables, the *T*-test for normally distributed continuous variables and the Mann–Whitney *U*-test for skewed variables. We used Spearman rank correlation to examine bivariate associations. Multiple linear regression was used to explore the association between vitamin D intake and serum 25(OH)D concentration. As vitamin D status did not differ between the two groups recruited to the study (i.e. the group with a high expected intake of contaminants and the reference group), all of the participants were initially combined in the data analyses. In the regression model, we adjusted for possible differences in sunlight exposure by including variables representing the month of blood sampling (March, April or May), and variables representing geographic location (Addresses were ordered into three groups according to latitude: Northern Norway, Mid-Norway and Southern Norway). Furthermore, covariates reported to be associated with vitamin D status were considered, and the following variables were included: sex, age, BMI, dietary retinol and dietary intake of dl-compounds (TEQ). Plasma triglycerides, plasma cholesterol, total energy intake, educational attainment and smoking status were also considered, but had no significant influence in any of the models.

All models were tested for interaction (on a multiplicative scale) by adding a cross-product of vitamin D intake and each of the covariates. A significant interaction between total intakes of vitamin D and retinol from food was seen, but when we stratified the group according to consumption (yes or no) of fish liver (as dinner or fish liver/roe pate') and/or seagull eggs, the interaction was no longer significant.

The assumptions of linearity and homoscedacity for all models were graphically tested by plotting predicted values against standardised residuals. We also applied Cook's distance and delta–beta plots to check the influence of outliers on the models. Statistical significance was determined by means of a two-sided probability level of $\leq$5% for the results and $\leq$10% for confounders. The statistical analyses were carried out using the statistical software PASW statistics 17 (SPSS, Chicago, IL, USA).

3 Results

3.1 Characteristics of the participants

The group with an expected high intake of contaminants was a little older, 59 versus 50 years ($p = 0.006$), and included a greater proportion of participants from the coast 55 versus 34% ($p = 0.008$) and Northern Norway 28 versus 18% ($p = 0.025$) than the reference group, whereas other socio-demographic factors were similar as well as the concentration of 25(OH)D (Supporting Information B). For the purpose of statistical analyses, the groups were therefore merged and analysed as a single group. Dietary supplements containing vitamin D (mainly in the form of cod liver oil), were taken by 108 (59%) of the participants.

3.2 Nutritional and toxicological exposures

The evaluation of nutritional and toxicological exposure for the total study population (Table 1) showed that 24 participants (13%) exceeded the TWI of dl-compounds [25], and that 16 participants (9%) exceeded a weekly intake of 400 g of oily fish [23] (http://www.vkm.no/dav/d94dff429b.pdf). A major feature of those who exceeded the TWI of dl-compounds was that all but one of them (95%) reported eating fish liver and/or seagull eggs, whereas 34% of the participants who did not exceed TWI for dl-compounds were consumers of fish liver and/or seagull eggs.

3.3 Stratification

Fish liver contains exceptionally high concentrations of vitamin D, retinol and dl-compounds, and is often eaten by the same individuals who eat seagull eggs. Seagull eggs, which are moderately high in vitamin D, contain exceptional high concentrations of dl-compounds in comparison with other food items. Consequently, we stratified the group of participants on the basis of consumption of fish liver and/or seagull eggs. There were significant differences between the strata with regard to participant' characteristics, seafood consumption, vitamin D, retinol and dl-compound intakes, and blood concentrations (Table 2).

3.4 Contributions of vitamin D and dl-compounds from different food groups

Only 13% of the group that did not eat fish liver and/or seagull eggs (13 out of 101 participants) met the recommended vitamin D intake (7.5–10 μg/day dependent on age) through food only. Another 39% (39 out of 101 participants) of the same group did this through food and vitamin D-containing supplements. Among the participants who did not consume fish liver and/or seagull eggs ($n = 101$), seafood contributed with 32% and the total contribution from seafood, fats (margarine, butter, oils and mayonnaise) and supplements were 93% of the total mean vitamin D intake. In this group, seafood was the main source of dl-compounds, contributing 54% of the mean intake (Fig. 1A), mainly from oily fish. The 5th and 95th percentiles of intake of oily fish were 15–390 g/wk, respectively. However, the median total intake of dl-compounds of this group was less than half the TWI of 14pgTEQ/kg b.w. [20] or 37%, whereas median total intake of dl-compounds of the group that ate fish liver and/or seagull eggs was 75% of the TWI. This was reflected in the median blood concentration of dl-compounds of the group that consumed fish liver and/or seagull eggs (Table 2), which was almost double that of the other group. Fish liver and seagull eggs contributed 34% of total intake of dl-compounds and 16% of total vitamin D intake in the group that consumed these foods (Fig. 1B).

3.5 Vitamin D intake and serum 25(OH)D concentration

Vitamin D intake was associated with serum 25(OH)D concentration in the group that did not consume fish liver and/or seagull eggs, but the association was weaker for the group

Table 1. Percentage of participants meeting or exceeding the recommended intake of vitamin D, vitamin D status, intake of oily fish or the TWI of dl-compounds

Meet the recommended intake of vitamin D[a)] μg/day	Vitamin D status 25(OH)D > 50 nmol/L[b)]	Food intake of oily fish > 400 g/wk[c)]	Contaminant intake TEQ/kg b.w. > 14pg/wk[d)]
59% ($n = 108/182$)	70% ($n = 128/182$)	9% ($n = 16/182$)	13% ($n = 24/182$)

a) Through food and supplements (7.5 μg/day for those < 60 years and 10 μg/day for > 60 years) [13].
b) Measured as 25-hydroxy-vitamin D [49].
c) Norwegian Scientific Committee for Food Safety [23].
d) Estimated as TEQs [20].

Table 2. Demographic characteristics, dietary exposures and concentrations of 25-hydroxy-vitamin D, retinol and dl-compounds in blood of participants stratified by consumption or no consumption of fish liver[a)] and/or seagull eggs

	Do not eat fish liver and/or seagull eggs ($n = 101$)	Eat fish liver and/or seagull eggs ($n = 81$)	p-Value
Gender – women	62%	46%	0.026
Education – university degree	47%	25%	0.003
Live inland	67%	38%	*<0.001*
Reference group	32%	46%	0.062
Current smokers	17%	36%	0.007
Live in Northern Norway	7%	47%	*<0.001*
Vitamin D supplement use	61%	56%	0.443
Seagull eggs			
1–5 per year	*0%*	*13%*	
>6 per year	*0%*	*14%*	
Age (years)	51 (28–75)	59 (34–76)	*0.002*
BMI (kg/m^2)	24 (20–32)	25 (21–32)	*0.004*
Amount seafood (g/day)	51 (19–125)	82 (36–170)	*<0.001*
Amount oily fish (g/day)	15 (2.1–56)	19 (7.0–91)	*0.001*
Amount lean fish (g/day)	16 (3.0–62)	33 (10–78)	*<0.001*
Amount liver (g/wk)	0	6.8 (0–31)	*<0.001*
Retinol from food (μg/day)[b)]	566 (274–1782)	936 (353–2290)	*<0.001*
Retinol from food and supplements (μg/day)[b)]	770 (340–2300)	1193 (498–2690)	*0.001*
Retinol in serum (μmol/L)	2.5 (1.7–4.0)	2.5 (1.7–3.4)	*0.613*
Vitamin D seafood (μg/day)	2.2 (0.5–6.7)	4.6 (2.1–12.0)	*<0.001*
Vitamin D from food (μg/day)	4.7 (1.7–10.4)	7.9 (3.3–16.1)	*<0.001*
Vitamin D from food and supplements (μg/day)	8.9 (2.7–26.8)	12.6 (4.2–27.8)	*0.001*
25(OH)D in serum (nmol/L)	62 (38–111)	56 (33–104)	*0.029*
Intake of dl-compounds (pg TEQ[c)]/kg b.w./wk)	5.2 (2.1–11.9)	10.5 (5.4–28.2)	*<0.001*
dl-Compounds in blood (pg TEQ[c)]/g lipid)	25 (12–66)[d)]	48 (21–117)[e)]	*<0.001*

Figures are given as percentages or the median (5th–95th percentiles).
a) As fish liver and/or fish liver/roe pate.
b) Not including calculated amounts available from carotenoids.
c) TCDD TEQs.
d) Blood values available for 21 participants.
e) Blood values available for 29 participants.

that did eat fish liver and/or seagull eggs (Table 3, Model 1). Adjusting vitamin D intake for intake of retinol in food increased the explained variance in 25(OH)D for the group that ate fish liver and/or seagull eggs (Model 2). The intake of dl-compounds was not significantly associated with 25(OH)D in this model. The intake of dl-compounds alone was not associated with serum 25(OH)D, neither in the group consuming fish liver and/or seagull eggs (−0.346; 95% CI: −0.918, 0.226) nor in the non-consumers (0.289; 95% CI: −1.42, 1.59). The intake of retinol and vitamin D from food in the whole group correlated strongly ($r_S = 0.53$, $p = 0.007$), which makes it difficult to disentangle the effects of retinol on 25(OH)D in the models (Table 3). However, using retinol intake from food as a substitute for vitamin D intake in the model, negatively influenced 25(OH)D in the group consuming fish liver and seagull eggs (−0.006; 95% CI: −0.021, 0.000), but not in non-consumers (0.002; 95% CI: −0.006, 0.011).

We further explored serum 25(OH)D concentration below and above the recommended level of 50 nmol/L stratified by consumption of fish liver and/or seagull eggs (Table 4). The main difference between those with a serum 25(OH)D concentration ≤ 50 nmol/L and those with a serum 25(OH)D concentration >50 was 5 mg/day (median) higher intake of supplementary vitamin D. Neither the intake of dl-compounds from food nor blood concentrations of dl-compounds differed between the high- and the low-25(OH)D strata for participants who did not eat fish liver and/or seagull eggs. Among participants who did eat fish liver and/or seagull eggs, the only significant difference between the low- and high-25(OH)D strata was as twice as high blood concentration of dl-compounds in the high 25(OH)D stratum (Table 4).

4 Discussion

Seafood high in vitamin D also contains dl-compounds and the content and balance between these might be crucial for the potential health benefit of fish [1, 3, 5]. We aimed to investigate whether the intake of vitamin D-rich foods resulted in unacceptably high intake of dl-compounds and whether the intake of dl-compounds affected the association between vitamin D intake and concentration of 25(OH)D. Our main findings were that supplemental vitamin D was an important contributor to adequate vitamin D status. Furthermore, all

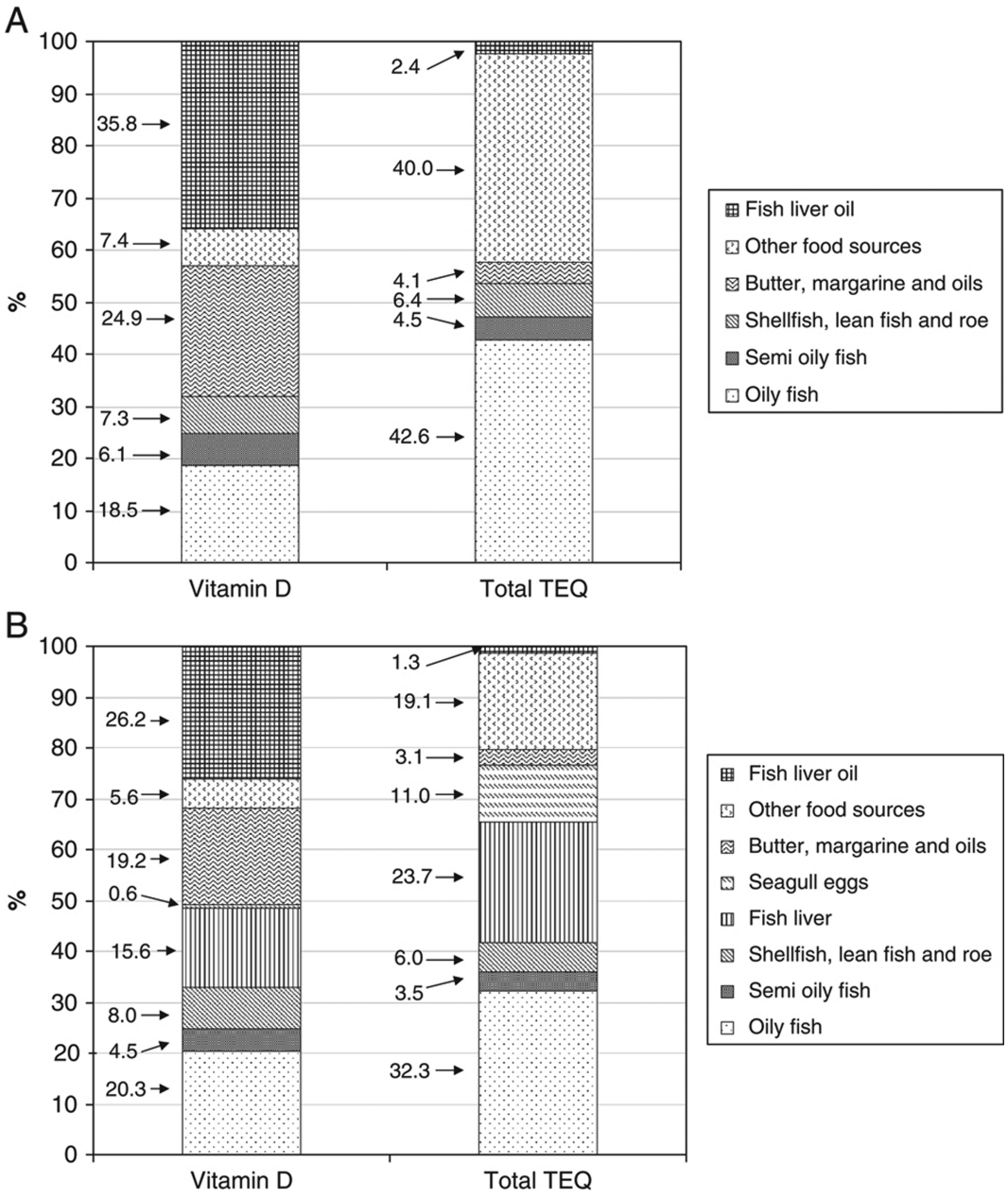

Figure 1. (A) Mean contribution of different food groups to intake of vitamin D (median = 8.9 μg/day) and dl-compounds (median = 5.2pg TEQ/kg b.w./wk). Data for non-consumers of fish liver and/or seagull eggs ($N = 101$). (B) Mean contribution of different food groups to intake of vitamin D (median = 12.6 μg/day) and dl-compounds (median = 10.5 pg TEQ/kg b.w./wk). Data for consumers of fish liver and/or seagull eggs ($N = 81$).

but one of the participants who exceeded the TWI was consumer of fish liver and sea gull eggs. The association between vitamin D intake and serum 25(OH)D seemed to be compromised, not by intake of dl-compounds, but possibly, through concomitant intake of retinol in participants who consumed fish liver.

The influence of retinol on the association between vitamin D intake and serum 25(OH)D pertained only to vitamin D derived from fish liver eaten as a meal or bread spread, not vitamin D contributed by commercial fish liver oil or other supplements (Table 3, Models 1 and 2). An intervention study ($n = 32$), in 1 wk providing 54 times the recommended daily dose of vitamin D through fish liver, found no increase in serum 25(OH)D concentrations, neither 12 h nor 4 days after the last meal [34]. The study was small and not randomized and most of the participants had an

Table 3. Linear regression[a)] between vitamin D intake from food and supplements and serum 25-hydroxy-vitamin D [25(OH)D] concentrations in participants stratified by no consumption or consumption of fish liver and/or seagull eggs

			(A) Do not eat fish liver and/or seagull eggs ($n = 101$)				(B) Eat fish liver and/or seagull eggs ($n = 81$)			
			β	(95% CI)	R^2	*p*-Value	β	(95% CI)	R^2	*p*-Value
Model 1	Vitamin D from food and supplements (μg/day)	Unadjusted	1.19	(0.65, 1.73)	0.16	<0.001	0.28	(−0.27, 0.83)	0.13	0.310
		Adjusted	1.06	(0.48, 1.63)	0.28	<0.001	0.52	(−0.05, 1.08)	0.17	0.072
Model 2	Vitamin D from food and supplements (μg/day)	Adjusted	1.03	(0.44, 1.62)	0.29	0.001	0.66	(0.12, 1.21)	0.26	0.018

Model 1: Adjusted for gender, age, BMI, geographic location and month of blood sampling. Model 2: Same as Model 1 but additionally adjusted for intake of retinol from food (μg/day) (Group A: retinol NS; Group B: retinol $p = 0.013$). Intake of dl-compounds was not significantly associated with serum 25(OH)D in this model.
a) The β-values denote the quantitative (nmol/L) increment in 25(OH)D expressed as nmol/L for each microgram increase in total vitamin D intake.

adequate vitamin D status at the start of the intervention. Another epidemiological study, including 443 women from Northern Norway, found a weaker association between vitamin D intake from fish liver and fresh fish liver oil and serum 25(OH)D than with vitamin D from other sources [35]. Fish liver contains exceptionally high concentrations of retinol [30] (www.matportalen.no/Matvaretabellen) (Accessed 2003), and the strengthening of the association between vitamin D intake and serum 25(OH)D concentrations when adjusting for retinol from food may point to possible interference of the nutrient in the absorption or metabolism of vitamin D (Table 3). The question of a potential retinol–vitamin D antagonism has been raised previously both in human studies [36–38] and in experimental animal studies [39–41] where an inverse association between high retinol intake and vitamin D status has been found. Retinol could interfere with absorption, transportation and conversion to the active form or the degradation of vitamin D. A mechanism might occur via competition between the active metabolites of retinol and vitamin D, retinoic acid (RA) and 1,25-hydro-xyvitamin D (1,25(OH)D), for the retinoic X receptor (RXR), which forms heterodimers both with the vitamin D receptor (VDR) and with the retinoic acid receptors (RAR), which could both influence expression of metabolizing enzymes and action in target cells [15, 42]. The association between dietary vitamin D and serum 25(OH)D was also influenced by the intake of dl-compounds although the association was weaker than for retinol. Activation of steroid and xenobiotic receptors by contaminants in fish liver and seagull eggs resulting in lower 1,25(OH)D concentrations should not be excluded as an explanation [15, 16].

The addition of retinol to refined commercial fish liver oil has been lowered deliberately by more than 50% over the last 20 years, as concerns over the possible adverse effects of the foetus and skeleton from a very high intake of retinol rose [43]. Furthermore, the amount of dl-compounds in commercial fish oil has been reduced to very low levels, whereas the added amount of vitamin D has been kept constant. Consequently, dietary supplements containing vitamin D such as cod liver oil are recommended to all population groups in Norway. In the present study, intake of 1 μg of vitamin D resulted in changes in serum 25(OH)D of 1.03 and 0.66 nmol/L, for non-consumers and consumers of fish liver and/or seagull eggs, respectively (Table 3). For comparison, these values bracket the steady state slope of 0.7 nmol/L increment in 25(OH)D for each additional 1 mg vitamin D input established as the quantitative relationship between vitamin D intake and serum 25(OH)D in an experimental study [44].

Fish liver and seagull egg consumption is rather uncommon among the general public in Norway. Hence, stratification provided an excellent opportunity to compare a group of people who eat these food items with a group of people with more common eating habits. The consumption of seafood by the group that did not eat fish liver and/or seagull eggs was similar to that found in earlier population studies [45]. Studies focusing on people living in Northern Norway, where fish liver consumption is more common, have not found a linear association between the intake of fish liver and the concentrations of dioxins and PCB in blood [46, 47]. However, in one of these studies, intake of other food items, such as oily fish, seagull eggs and crude fish liver oil, does not seem to have been taken into consideration [46], making interpretation hard. The study by Rylander et al. [47] found crude fish oil and seagull eggs, but not fish liver per se, to be the most significant predictors of the concentration of three of the most common PCBs. The content of dl-compounds in fish liver varies, with a lower content being found in fish caught in Northern Norway [48] (http://www.nifes.no/index.php?page_id=&article_id=3442&lang_id=2). The results are therefore not inconsistent with the results presented here. It is important to point out that the TWI for contaminants incorporates a safety margin and is not a threshold for adverse effects on health. Exceeding the TWI, therefore, represents an erosion of the safety margin. However, the results clearly show that consumption of fish liver and/or seagull eggs can bring the intake considerably closer to or even above the TWI than if these food items are not consumed.

Table 4. Dietary exposure of vitamin D and serum concentrations of 25-hydroxy-vitamin D [25(OH)D], retinol and dl-compounds in blood, geographic location and time of blood sampling stratified by fish liver and/or seagull egg consumption and serum 25(OH)D levels below or above 50 nmol/L

	Do not eat fish liver and/or seagull eggs ($n = 101$)			Eat fish liver and/or seagull eggs ($n = 81$)		
	≤50 nmol/L 25(OH)D $n = 25$ (25%)	> 50 nmol/L 25(OH)D $n = 76$ (75%)	*p*-Value	≤50 nmol/L 25(OH)D $n = 29$ (36%)	>50 nmol/L 25(OH)D $n = 52$ (64%)	*p*-Value
Use supplement containing vitamin D	11 (18%)	51 (82%)	0.040	14 (30%)	32 (70%)	0.248
Achieve vitamin D recommendation	7 (13%)	45 (87%)	0.007	17 (30%)	39 (70%)	0.126
Exceed TWI for total TEQ intake	0	1 (100%)	–	8 (35%)	15 (65%)	0.904
Vitamin D	Median (P5–P95)	Median (P5–P95)		Median (P5–P95)	Median (P5–P95)	
Vitamin D from food (μg/day)	4.3 (0.6–9.2)	4.8 (1.7–10.8)	0.072	7.3 (2.2–30.3)	8.8 (3.9–19.7)	0.054
Vitamin D from seafood (μg/day)	2.1 (1.1–7.2)	2.4 (0.5–7.3)	0.571	4.3 (1.1–21.0)	4.8 (2.1–14.7)	0.162
Vitamin D from oily fish (μg/day)	1.2 (0–4.9)	1.4 (0.1–5.2)	0.469	1.5 (0.4–6.3)	1.9 (0.8–7.4)	0.059
Vitamin D from fish liver (μg/day)	0	0		1.3 (0–24.3)	1.1 (0–8.7)	0.708
Vitamin D from fat (μg/day)	1.2 (0.2–4.0)	2.1 (0.4–4.4)	0.036	1.8 (0.4–6.2)	2.3 (0.3–5.8)	0.073
Vitamin D from other sources (μg/day)	0.3 (0.1–1.6)	0.3 (0.1–2.4)	0.368	0.3 (0.1–2.4)	0.3 (0.1–4.5)	0.906
Vitamin D from supplements (μg/day)	0 (0–9.4)	5.0 (0–21)	0.002	0 (0–15.4)	1.4 (0–19.0)	0.204
Serum 25(OH)D (nmol/L)	40 (20–50)	68 (51–117)	<0.001	42 (22–50)	62 (52–113)	<0.001
Retinol						
Retinol from food (μg/day)	474 (146–2093)	627 (271–1866)	0.094	1,019 (362–4310)	909 (309–2406)	0.165
Retinol from food and supplements (μg/day)	596 (198–2127)	909 (366–2567)	0.007	1235 (421–4328)	1191 (464–2681)	0.448
Retinol in serum (μmol/L)	2.2 (1.6–3.7)	2.5 (1.7–4.1)	0.202	2.3 (1.5–4.2)	2.5 (1.7–3.3)	0.602
Total TEQ[a)]						
Total TEQ[a)] pg/kg b.w./wk from food	5.2 (1.5–13.3)	5.4 (2.1–12.1)	0.418	10.5 (4.2–47.8)	10.4 (5.5–29.2)	0.581
Total TEQ[a)] in blood (pg/g lipid)	24 (8–66.0) ($n = 8$)	25 (12-72) ($n = 13$)	0.972	27 (22–96) ($n = 6$)	61 (18–131) ($n = 23$)	0.031
Geographic location (North = 1, Middle = 2, South = 3)	3 (1–3)	3 (2–3)	0.745	2 (1–3)	2 (1–3)	0.901
Month of blood tests sampling (March = 3, April = 4, May = 5)	4 (3–5)	4 (3–5)	0.572	4 (3–5)	4 (3–5)	0.161

Values shown as percentages and the median (5th–95th percentiles).
a) Toxic equivalents.

Another finding of the study was that in the group that did not consume fish liver and/or seagull eggs, the total intake of dl-compounds was low, despite moderate to high intake of oily fish (Table 4). However, the main difference between subjects in this group, when studying those with serum 25(OH)D concentrations above and below 50 nmol/L, was their vitamin D intake from supplements. As only 13% of participants in this group obtained the recommended intake of vitamin D through food alone, supplements critically contributed to an adequate vitamin D status for a majority of the participants not consuming fish liver and/or seagull eggs. Intake above the recommended intake level has been assumed to ensure 25(OH)D above 50 nmol/L [49] (http://www.helsedirektoratet.no/vp/multimedia/archive/00013/IS-1408_13064a.pdf). Concurrently, the majority of participants in this group who met the recommended intake of vitamin D from food and supplements showed an adequate serum 25(OH)D concentration, without exceeding the TWI for dl-compounds.

The mean intake of dl-compounds in the group not consuming fish liver or seagull eggs was far below the TWI of 14pgTEQ/kg b.w. set by the European Scientific Committee for Food, even among those who met the vitamin D recommendation (results not shown). Studies investigating population sub-groups with high fish consumption have not found the intake of fatty fish to significantly affect the body burden of POPs [47]. The results of this study suggest that there is room for an even higher intake of oily fish than the median of 105 g/wk observed in this study, which is considerably lower than 400 g/wk suggested by the Norwegian Scientific Committee for Food Safety [23] (http://www.vkm.no/dav/d94dff429b.pdf). Since the NFG Study was performed in 2003, the average concentrations of dl-compounds in farmed fish have been halved. Today, they are considerably lower than the concentrations found in wild fish [48] (http://www.nifes.no/index.php?page_id=&article_id=3442x&lang_id=2).

The main strengths of this study include the detailed dietary information, the biological measurement of nutrients and toxic compounds, and the inclusion of participants with widely differing intakes of food known to be high in contaminants [25]. The participants included men and women from a wide geographic area, who reported diverse dietary habits and socio-demographic differences. A novel feature of this study was the incorporation of an extensive database of dioxins and PCBs to estimate the total dietary intake of dl-compounds [25]. However, several factors might distort our findings. No dietary assessment methods are without error [27]. In addition to the seasonal and regional variations in food traditions, regional variation in vitamin D and dl-compound concentrations may further contribute to imprecision in estimated exposure levels for population sub-groups. Most dietary surveys have limitations when it comes to monitoring environmental contaminants, as they usually focus on the general population and regularly eaten food items, rather than on food, often seasonal (e.g. seagull eggs, fish liver and fish liver pâté), eaten only by sub-groups [48] (http://www.nifes.no/index.php?page_id=&article_id=3442&lang_id=2).

In conclusion, the association between vitamin D intake and serum 25(OH)D did not seem to be compromised by dl-compounds. This study shows that the best way of achieving an adequate vitamin D status while keeping the concentrations of dioxins and dl-PCBs low is to consume moderate amounts of a variety of oily fish types as part of a healthy diet, restrict intake of highly contaminated food and use vitamin D supplements. Our results support present-day dietary advice that intake of fish liver and seagull eggs should be restricted, despite high nutrient content, both because of high content of dl-compounds and possible vitamin-A-D antagonism.

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