Five salmon dinners per week was not sufficient to prevent the reduction in serum vitamin D in autumn at 60º north latitude: a randomised trial

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Conflict of interest Rosenlund and Sveier are employed in Skretting Aquaculture Research Centre AS and Lerøy Seafood Group ASA, respectively. Skretting Aquaculture Research Centre AS is a global leader in providing innovative and sustainable nutritional solutions for the aquaculture industry. Lerøy Seafood Group ASA is the leading exporter of seafood from Norway and the world's second largestproducer of Atlantic Salmon. Skretting Aquaculture Research Centre AS and Lerøy Seafood Group ASA were not involved in on-site data collection. The other authors declare no conflicts of interest.

Abstract

Low serum concentrations of several vitamins have been linked to increased risk of diseases including insulin resistance and type 2 diabetes (T2D). Fish is a good source of several vitamins, and the prevalence of T2D is low in populations with high fish intake. The aim of this study was to investigate the effects of high intake of cod or salmon on a comprehensive panel of vitamins in serum from adults with overweight/obesity in autumn in South-Western Norway at 60º north latitude. In this randomised clinical trial, sixty-three healthy participants with overweight/obesity consumed 750 g/week of either cod (N=22) or salmon (N=22) as 5 weekly dinners, or were instructed to continue their normal eating habits but avoid fish intake (Control group, N=19) for 8 weeks. The estimated vitamin D intake was significantly increased in the Salmon group when compared to the Cod group ($P=6.3x10^{-4}$) and to the Control group ($P=3.5x10^{-6}$), while for vitamins A, B1, B2, B3, B6, B9, C and E, no differences in estimated intake were found between groups. Serum 25-hydroxyvitamin D3 concentration was decreased in all groups after 8 weeks, however, the reduction in Salmon group was significantly smaller compared to both the Cod group (P=0.013) and the Control group (P=0.0060). Cod and salmon intake did not affect serum concentrations of the other measured vitamins. The findings suggest that 750 g/week of salmon was not sufficient to prevent a decrease in serum 25-hydroxyvitamin D3 in autumn in South-Western Norway in adults with overweight/obese.

Introduction

Fish is a good dietary source of nutrients such as proteins, fats, and several vitamins and minerals; however, the contents of nutrients in fish varies across fish species [1]. Fish consumption is associated with reduced risk of diseases such as type 2 diabetes and cardiovascular disease [2-8], and the health benefits of fish consumption have traditionally been attributed mainly to the effect of n-3 polyunsaturated fatty acids and vitamin D, especially from fatty fish. Vitamins play essential roles in numerous processes in the body, including metabolism of carbohydrates, fatty acids and amino acids [9-15]. Low serum concentrations of fat-soluble and water-soluble vitamins have been linked to the risk of developing obesity related co-morbidities including insulin resistance and type 2 diabetes, and low circulating concentration of 25-hydroxyvitamin D3 is associated with increased risk of cardiovascular events [16, 17]. In Norway, vitamin fortification is limited to dairy products (vitamin D) and margarine (vitamins A and D) [18]. Cutaneous synthesis of 25-hydroxyvitamin D3 stimulated by sunlight is the major source of vitamin D in humans [19]; therefore, vitamin D-rich foods such as fatty fish, seafood, cod-liver oil, entrails and fortified products are important food sources in northern countries such as Norway in late autumn, winter and early spring when UVB exposure is low.

We have previously demonstrated that a high salmon intake (750g/week) for eight weeks improved postprandial glucose regulation in study participants with overweight/obesity, whereas high cod intake (750g/week) did not affect glucose regulation in the same study setting [20]. In the present study we wanted to further explore the biological materials from this randomized clinical trial to gain insight into if a high intake of cod or salmon would affect serum vitamin status. The aim of the present study was to investigate effects of high intake of salmon or cod on serum vitamin status. The intervention was conducted in the autumn of 2011 in South-Western Norway (60º north latitude). Our hypothesis was that the high salmon intake would prevent the normal seasonal decline in circulating vitamin D concentration in autumn, and that high intake of cod or salmon would improve the general vitamin status.

Methods

Participants, study setting and ethics

The study design, the description of study participants, the study setting and the protocol for study visits have been published in detail previously [20]. In brief, the study population consisted of adults with overweight or obesity, and all participants were of Norwegian ethnic origin (Caucasian) living in the Bergen area in South-Western Norway at 60º north latitude. Inclusion criteria were; BMI ≥27 kg/m2, fasting blood glucose ≤7·0 mmol/l and age 18–69 years. Exclusion criteria were high habitual fish/seafood intake (> 500g/week), pregnancy, incompatibility with fish consumption (allergies, intolerance and/or dislike), diagnosed diabetes mellitus, heart disease or gastrointestinal diseases, use of medications affecting lipid metabolism or glucose homoeostasis, use of anti-inflammatory

medications, use of supplements containing n-3 PUFAs, intentional weight loss and large fluctuation in body weight (>3 kg) over the previous 2 months. Participants were interviewed about their fish/seafood intake before they were included in the study, and those with a regular fish intake > 1 fish dinner per week were instructed to avoid eating fish for 4 weeks before the baseline visit.

The study was designed as a randomised, controlled intervention study with a parallel group design, with three intervention arms: Atlantic cod (wild-caught *Gadus morhua*) in weekly doses of 750 g, salmon (farmed *Salmo salar*) in weekly doses of 750 g, and a no-fish group as the Control group. The intervention period was 8 weeks. In all, seventy-six participants were included in the study and were randomly assigned to the Cod group (*n* 27), Salmon group (*n* 27) or the Control group (*n* 22). The participants were randomised into the different groups by the project manager by drawing lots, and the participants were informed about their group allocation during the baseline visit. All examinations were conducted at the Clinical Research Unit at the Haukeland University Hospital, Bergen, Norway. To enhance compliance the participants were contacted by phone approximately one week prior to baseline and end point visits, during which they were informed of the schedule and procedures for the following visit. Also, a text message was sent 1-3 days before the 8 week visit, as a reminder of how to prepare for the upcoming visit. For any inquires during the trial period, members of the research group could be reached by email or telephone. Compliance was monitored through interviews; after one, four and eight weeks intervention the participants in the fish eating groups were asked how many dinners with cod/salmon they had not eaten since last contact, instead of asking how well they had complied, to lower the bar for reporting missing intake. Noncompliance was defined as not following the protocol in regard to fish intake (omitting more than 3 fish dinners in the fish eating groups), other dietary changes or use of prescription medicine not compatible with the inclusion criteria, or changes in physical activity. As reward for completing the study, participants were offered a dietary consultation with a student dietician at the last visit and all the results of analyses of blood samples.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures were approved by the Regional Committee for Medical and Health Research Ethics of Western Norway (REC no.: 2011/572). Written informed consent was obtained from all subjects.

Health professionals performing blood sampling, and personnel conducting the laboratory analyses, were all blinded to participants' identity and group allocation. All data were analysed anonymously. This trial was registered at clinicaltrials.gov as NCT02350595.

Interventions

Cod and salmon fillets were provided to the participants as frozen-skin and boneless-fillet portions (mean weight with standard deviation; 150 (SD 10) g; Lerøy Seafood Group ASA), and pallets of fish were chosen at random from Lerøy's warehouse in Bergen, Norway. The cod and salmon fillets were supplied free of charge to the participants, and were distributed at the baseline visit or at any time

during the study period, if preferred. Participants in the Cod group were instructed to eat five dinners per week containing 150 g of cod fillet, and participants in the Salmon group were instructed to eat five dinners per week containing 150 g of salmon fillet. The participants in the fish-eating groups were told not to exceed a total amount of 750 g of fish/week, not to consume any other types of fish or seafood during the study period, and to maintain their normal eating habits throughout the study period apart from eating the mandatory amount of 750 g fish/week. The Control group was also instructed to continue their normal eating habits, except to avoid fish and seafood intake. Subjects in all groups were instructed not to change their physical activity level during the intervention period. The participants' dietary intake and habitual lifestyle were recorded at the baseline and the endpoint visits, using food record charts and a questionnaire for reporting physical activity. Reported energy and macronutrient intake and physical activity were not changed within the groups during the study period [20].

Protocol for study visits

The total study period was 8 weeks, with baseline visits between August 22, 2011 and September 19, 2011. Examinations were conducted in the morning after an overnight fast. The subjects were instructed not to eat or drink anything except water, and not to use substances containing nicotine after 22.00 hours the previous day, and to avoid physical exercise and alcohol for 24 h before each sampling day.

Body height was measured at the baseline, using a wall-mounted stadiometer (Seca 222; Seca). Body weight and body composition were measured in a fasted state using a bioelectrical impedance analysis device (InBody 720; Biospace Co. Ltd) at the baseline and the endpoint.

Fasting blood samples were collected at the baseline and the endpoint, in BD Vacutainer SST II Advance gel tubes (Becton, Dickinson and Company) for isolation of serum. The staff complied with a strict protocol for pre-analytical sample handling to ensure high sample quality. Blood samples were centrifuged after 30 minutes at room temperature, and serum were immediately aliquoted and frozen at -80°C until analyses.

Estimation of vitamin intakes from dietary records

Participants completed dietary records of the 5 preceding days before the baseline visit and the 5 preceding days before the 8-week visit, including at least 1 weekend-day. The intakes of vitamins A, B1, B2, B3, B6, B9, C, D and E were calculated from the participants' dietary records using the 'Mat på Data 5.1' software [21], which contains information on the nutrient contents in food items sold in Norway. This food data base does not contain information of vitamins B12 and K1 contents in foods. Food records were checked for completeness at both study visits.

Analyses of serum samples

Vitamin B1 (thiamine and thiamine monophosphate), vitamin B2 (riboflavin and flavin mononucleotide), vitamin B3 (nicotinic acid, nicotinamide and N1-methylnicotinamide), vitamin B6 (pyridoxal 5'-phosphate, pyridoxal, pyridoxine and 4-pyridoxic acid) were analysed in serum by Bevital AS (Bergen, Norway, http://www.bevital.no) using liquid chromatography combined with tandem mass spectrometry, as previously described [22]. Thiamine, thiamine monophosphate, nicotinic acid, nicotinic acid, nicotinamide and N1-methylnicotinamide with corresponding isotope labelled internal standards were added to the previously published assay [22]. Vitamin A (all-trans retinol), vitamin D (25-hydroxyvitamin-D2 and 25-hydroxyvitamin D3), vitamin E (alpha-tocopherol and gamma-tocopherol), and vitamin K1 (phylloquinone) were measured by liquid-chromatography-tandem mass spectrometry [23], and vitamin B12 [24] and folate [25] by microbiological assays. Serum concentration of vitamin K1 was below level of detection (0.33 nmol/l) for 16 samples, for these we used value level of detection divided by 2. Serum concentrations of 25-hydroxyvitamin D2, nicotinic acid and pyridoxine were below level of detection in all samples.

Outcome measurements

The primary outcome of the present study was changes in serum vitamin D concentration after a weekly intake of 750 g fillet from either salmon or cod for 8 weeks. Secondary outcomes were changes in serum concentrations of other fat-soluble and water-soluble vitamins, and changes in estimated dietary intakes of vitamins within the groups over time.

Sample size estimation

The sample size calculation for this trial was originally conducted with the aim to investigate the effects of high intake of cod or salmon on postprandial glucose regulation after a standardised breakfast in participants with overweight or obesity [20]. We estimated that it was necessary to include 76 participants divided into three groups to ensure that 20 participants in each group completed the trial with satisfactory compliance, with a power of 80% and α of 0.05, and of these 65 participants were included in statistical analyses [20]. In the present study we wanted to further explore the biological materials from this randomised clinical trial to investigate if a high intake of cod or salmon would affect serum vitamin status. The primary aim of the present study was to investigate the effects of high intake of salmon or cod on changes in serum vitamin D concentration in autumn at 60º north latitude. From two of the participants we did not have a sufficient amount of blood serum left for analyses, thus we had serum samples from sixty-three subjects available for laboratory and statistical analyses.

This is the first study to investigate the effects of 8 weeks of high intake (750g/week) of cod or salmon on serum vitamin status in adults with overweight/obesity living in South-Western Norway, therefore no data are available for sample size calculation for the present study. However, sample size

estimation using data from a pilot study conducted in spring in Bergen, Norway with normal-weight adults consuming 750g/week of cod, salmon or chicken for 4 weeks showed that 13 participants in each group was sufficient to detect differences between groups for changes in vitamin D, with a power of 80% and α of 0.05. Based on this, the present study where 19-22 adults with overweight/obesity consume 750g/week of cod, salmon or fish-free diet for 8 weeks should have the sufficient statistical strength for comparing changes in vitamin D in autumn at 60º north latitude between groups consuming cod, salmon or fish-free diet.

Statistical analyses

Statistical analyses were conducted using SPSS Statistics 25 (SPSS, Inc., IBM Company). Subjects who did not complete the study were excluded from the statistical analyses. For analytes in serum and for estimated intake of vitamins from dietary records, most data were not normally distributed according to the Shapiro–Wilk test, and nonparametric tests were used to investigate changes within groups (the Wilcoxon-signed ranks test). For these non-parametric data, the Kruskal–Wallis Test was used to compare values between the three groups at baseline. Changes within the groups were compared using univariate analysis of covariance (ANCOVA) with adjustment for baseline values after log transformation, followed by the Tukey's Honestly Significant Difference (HSD) Test whenever between-group differences were detected. Data are expressed as medians and 25th, 75th percentiles. Categorical data were compared using the Pearson's χ^2 test. All comparisons were two-sided, and $P<0.05$ was considered statistically significant.

Results

Participant characteristics

In total, seventy-six participants were included in the study and completed the first study visit, and sixty-eight participants completed the trial. One participant (a woman in the Salmon group) was excluded from statistical analysis after analyses of postprandial blood glucose revealed she had prediabetes, and two participants (one woman in the Cod group and one man in the Salmon group) were withdrawn from analysis because they did not comply with the protocol. From two of the participants (one man in the Salmon group and one man in the Control group), we did not have a sufficient amount of blood serum for analyses, therefore these two subjects are excluded from all analyses in the present paper. In total, sixty-three subjects (twenty-seven men and thirty-six women) were included in the statistical analyses. The flow of participants in the study is presented in **Figure 1**.

Groups were similar at baseline in regard to gender distribution, age, BMI, percentage body fat or percentage muscle mass (**Table 1**), with median age 45.6 (25th,75th percentile 37.1,53.9) years and median BMI 32.3 (25th,75th percentile 29.6,35.7) kg/m^2. After 8 weeks, no changes were seen in any of the groups for BMI, percentage body fat or muscle mass (data not presented).

Estimated dietary intake of vitamins

The participants registered food intake for the last five days before the endpoint visit. The Cod group reported 71% of dinners to contain cod, whereas 16% of the meat for dinners was sausages, minced meat, hamburger and pizza/lasagne with meat, 4% of dinners contained chicken, 4% contained lamb, with only one reported dinner was meat-free. In the Salmon group, 71% of dinners contained salmon, whereas 15% contained meat from sausages, minced meat, hamburger and pizza/lasagne with meat, 6% of dinners contained pork, 4% contained chicken, and no meat-free dinners were reported. Participants in the Control group, which were not allowed to eat fish or seafood during the intervention period, preferred to include meat as part of their dinners; of the 95 dinners registered by the 19 participants in the control group, only one of the reported dinners did not contain meat. The most popular types of meat for dinner in the Control group were sausages, minced meat, hamburger and pizza/lasagne with meat (a total of 48% of the dinners), followed by pork (17% of dinners) and chicken (12% of dinners). None of the participants in any of the three groups reported intake of liver or kidney in their food diaries before the baseline and endpoint visits.

The estimated median intakes of vitamins A, B1, B2, B3, B6, B9, C, D and E were similar between the groups at baseline (Kruskal-Wallis test, $P>0.05$). The estimated vitamin D intake was significantly increased from baseline to 8 weeks in the Salmon group when compared to both the Cod group and the Control group (**Table 2**). For the other vitamins, no changes were seen in estimated daily intake within any of the groups from baseline to 8 weeks.

Vitamins in serum

Concentrations of all-trans retinol, thiamine, thiamine monophosphate, riboflavin, flavine mononucleotide, nicotinamide, N^1-methylnicotinamide, pyridoxal 5'-phosphate, pyridoxal, 4-pyridoxic acid, folate, cobalamin, 25-hydroxyvitamin D3, alpha-tocopherol, gamma-tocopherol and phylloquinone in serum were similar between the groups at baseline (Kruskal-Wallis test, $P>0.05$) (**Table 3**).

Serum 25-hydroxyvitamin D3 concentration was significantly reduced within all groups from baseline to endpoint, however the reduction in vitamin D3 in the Salmon group was significantly smaller when compared to both the Cod group and the Control group (**Figure 2**). Otherwise, no changes were seen in serum concentrations for the other vitamins during the intervention period (Table 3).

Discussion

In the present study we investigated the effects of high intake of cod or salmon fillets on serum concentrations of a comprehensive panel of water- and fat soluble vitamins. We observed that a

weekly intake of 750 g salmon fillet curbed but was not sufficient to prevent the expected seasonal decrease of serum 25-hydroxyvitamin D3 in the autumn in Norway.

We have previously demonstrated that a high salmon intake (750g/week) improved postprandial glucose regulation in these study participants, whereas high cod intake (750g/week) did not affect glucose regulation after 8 weeks intervention [20]. The estimated intakes of energy and macronutrients (protein, fat and carbohydrates) as well as estimated physical activity were similar between the experimental groups at baseline and did not change from baseline to endpoint [20]. In the present paper we extended the statistical analyses of estimated intakes and show that no differences were seen between the groups for estimated intakes of vitamins A, B1, B2, B3, B6, B9, C, D and E at baseline, and of these vitamins, only vitamin D estimated intake increased from baseline to endpoint in the Salmon group when compared to the Cod group and the Control group. The presented findings regarding estimated intakes of energy, macronutrients [20] and vitamins based on food diaries gives a good indication that the participants did not change their dietary habits during the study period, except for the inclusion of cod or salmon in the fish eating groups.

In humans, cutaneous synthesis of 25-hydroxyvitamin D3 stimulated by sunlight is the major source of vitamin D [19]. The participants in the present study were living in Bergen, in South-Western Norway, at 60º north latitude. The baseline visits were in the last week of August and the three first weeks of September, with the endpoint visits starting in the middle of October and lasting throughout the first third of November. The study was thus conducted in a period when Bergen usually experiences a lot of overcast weather with little sun, and the inhabitants have little chance of getting direct sunshine on their skin for cutaneous synthesis of vitamin D3. The autumn of 2011, when the current study was conducted, was no exception with 4 days of fair weather and 64 rainy days with a total of 783 mm rainfall during the 80 days of the study period [26]. A reduction in circulating vitamin D is expected in the autumn this far north of the Equator since the intensity of the UVB radiation is too low for subcutaneous production of vitamin D [27, 28], unless the vitamin D intake from food or supplements is increased. The Nordic Council of Ministers recommends daily intake of 10 µg vitamin D for the adult Nordic population (<75 years), and recommends circulating 25-hydroxyvitamin D concentrations ≥ 50 nmol/L [29]. At baseline, 50 of the 63 participants (84%) had serum 25-hydroxyvitamin D3 concentration > 50 nmol/L, but 8 weeks later this was seen in 49% of the participants (31 participants). Fatty fish such as salmon is regarded to be a good dietary source of vitamin D3 [1, 21], and we expected that the high intake in the Salmon group of this study would be sufficient to sustain the serum 25-hydroxyvitamin D3 concentration throughout autumn. However, although the median daily vitamin D intake was estimated to be 11.9 µg (interquartile range 10.0,14.0 µg) in the Salmon group, which was above the recommendation of 10 µg vitamin D per day, the serum 25-hydroxyvitamin D3 concentration decreased in this group, albeit to a lesser degree when compared to both the Cod group and the Control group. Thus, a weekly intake of 750g salmon was insufficient to

prevent the seasonal reduction in serum 25-hydroxyvitamin D3 in the autumn in this group of healthy adults with overweight or obesity living in South-Western Norway.

The contents of vitamin A, vitamin B1, niacin and vitamin B6 are higher per wet weight in salmon fillet compared to cod fillet, whereas amounts of vitamin E, vitamin B2, folate and vitamin C are comparable in cod and salmon fillet [21]. We found no differences in the estimated dietary intakes of these 8 vitamins when comparing the food diaries from the Cod group, the Salmon group and the Control group. The participants had a varied diet throughout the study period, and regularly reported that they consumed common foodstuffs that are good sources of different vitamins, such as dairy products (vitamins A and B2, folate), egg (vitamins A, B1, B2, E and folate), fruits (vitamin C, folate), vegetables (vitamins A and C, folate), meat (vitamins A, B1, B2 and B6, niacin, folate), grains (vitamins B1, B2 and B6, folate, niacin), nuts (vitamins B1, B2, B6 and E, niacin, folate), legumes (vitamins A, B1, B2 and C, folate) and berries (vitamins C and E, folate) [21]. In line with this, no changes in serum concentrations of vitamins A, B1, B2, B6, E, and niacin and folate were found within or between any of the experimental groups.

Vitamin K1 and cobalamin are not included in the food database (Mat på data) used for estimations of dietary intakes in the present study, therefore we were not able to report estimated intake of these vitamins. Little information is available on the content of vitamin K1 in various foods, but this vitamin is found in highest amounts in plants and vegetable oils [30], and no changes were seen in serum vitamin K1 concentration in any of the groups during the intervention. Only a few food types are rich in vitamin B12, such as fish, meat, egg and dairy products [30], which were regularly consumed by our participants, in addition to liver and kidney [30] which were not reported in the food diaries by any of the participants in the present study. The bioavailability of vitamin B12 is also an issue when considering food sources of this vitamin, and it has been reported that for humans the bioavailability is several times higher from milk, fish and meat compared to egg [31]. The cobalamin content in farmed Atlantic salmon is approximately three times that in cod [1], but still no differences were observed between the groups for changes in serum cobalamin concentrations after 8 weeks intervention.

This study has some strengths and limitations. Strengths include the measurement of both water- and fat-soluble vitamins, and the use of 5-days dietary registrations at baseline and endpoint for estimation of vitamin intake. A possible limitation of the present study is that the results obtained may not be valid for individuals with different demographics such as BMI, living at different latitudes, and different lifestyles than those in the present work, thus additional studies are needed to address these factors. Also, the effects of a longer intervention period on the serum micronutrient concentrations should be investigated. The vitamin D serum concentration for the participants that habitually consumed >1 fish dinner per week may have been lower in the beginning of study than normal since these participants had a 4 week fish-free period before the baseline visit. The cod and salmon fillets used in the present study were randomly chosen from different batches in Lerøy's warehouse in Bergen, and were representative for cod and salmon sold in Norwegian grocery stores. We did not

conduct our own analyses of vitamin contents in these batches, but instead we used average values from the official Norwegian food database.

Conclusion

A high intake of salmon curbed but was not sufficient to prevent a decrease in serum 25-hydroxyvitamin D3 concentration in autumn in South-Western Norway in adults with overweight/obesity. We also found that high intake of cod or salmon did not affect serum concentrations of the other investigated vitamins. The Norwegian Directorate of Health recommends a weekly intake of 300-450 g fish, of which at least 200 g should be fatty fish, for the general public [32], however findings in the present study suggest that such intake will not be sufficient to prevent the decline in serum vitamin D concentration in autumn at 60º north latitude. This result suggests that it is difficult to sustain non-deficient levels of vitamin 25- hydroxyvitamin D3 in autumn in South-Western Norway through dietary intake alone.

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G. R. and H. S. are employed in Skretting Aquaculture Research Centre AS and Lerøy Seafood Group ASA, respectively. Skretting Aquaculture Research Centre AS is a global leader in providing innovative and sustainable nutritional solutions for the aquaculture industry. Lerøy Seafood Group ASA is the leading exporter of seafood from Norway and the world's second largest producer of Atlantic Salmon. Skretting Aquaculture Research Centre AS and Lerøy Seafood Group ASA were not involved in on-site data collection. The other authors declare no conflicts of interest.

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Figure legend

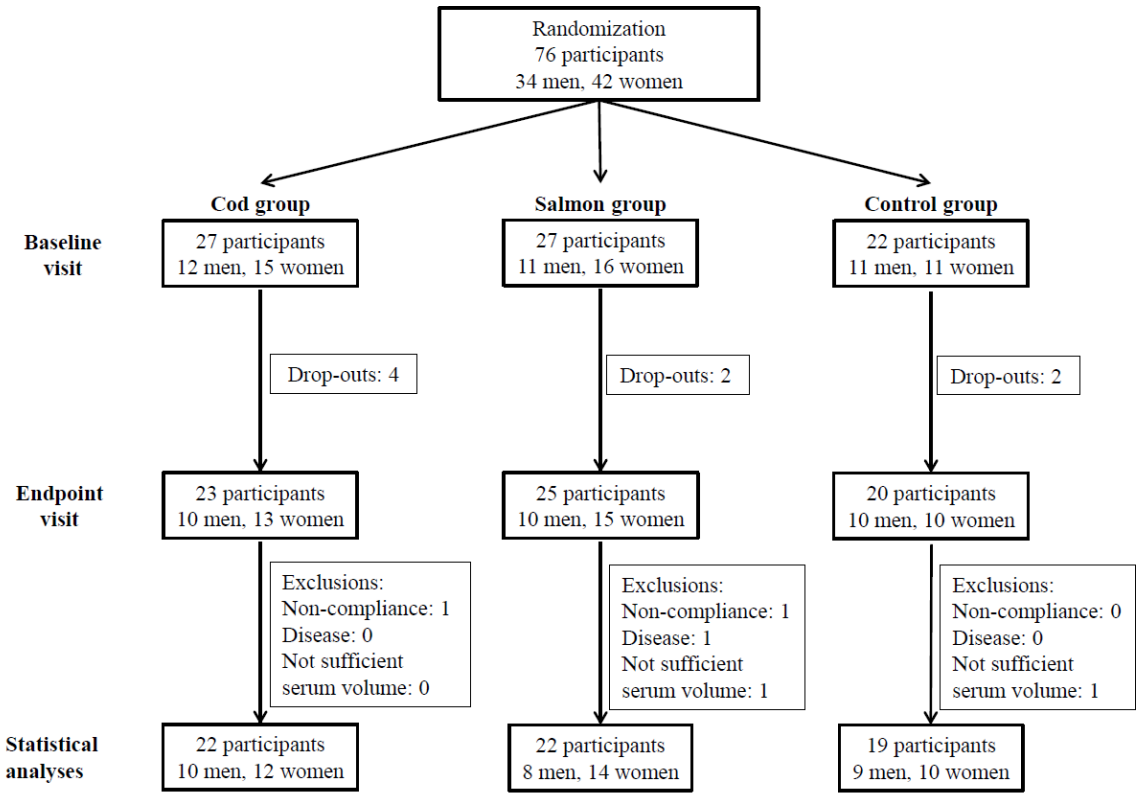

Figure 1

Flow diagram displaying the progress of participants during the study period. Participants who did not comply with the study protocol were excluded from statistical analysis. Noncompliance was defined as not following the protocol in regard to fish intake (omitting more than 3 fish dinners in the fish eating groups), other dietary changes or use of prescription medicine not compatible with the inclusion criteria, or changes in physical activity.

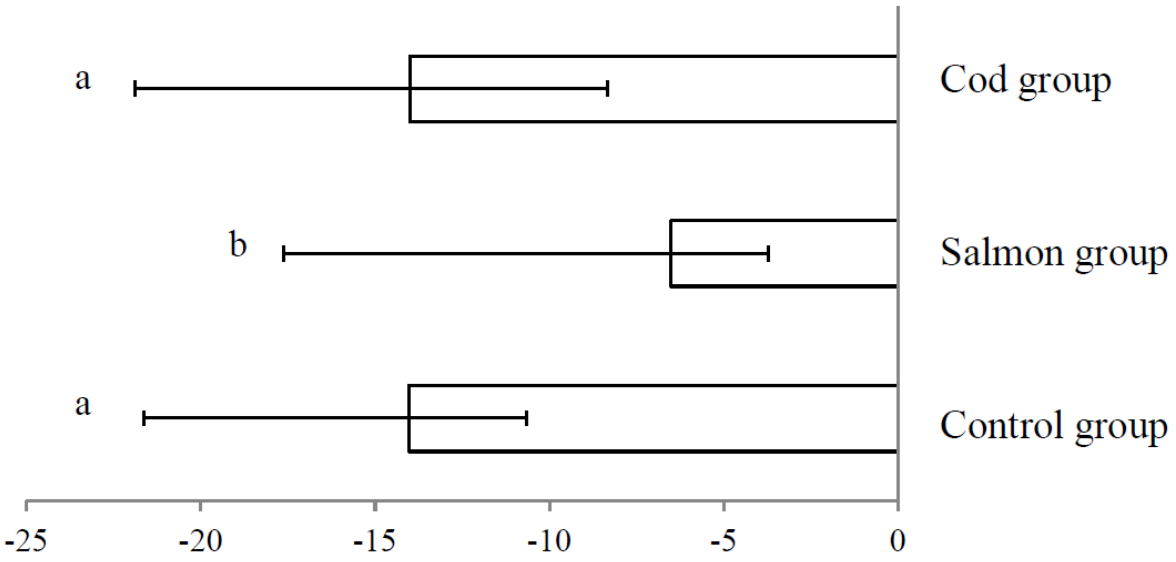

Figure 2

Change in serum concentrations of 25-hydroxyvitamin D3 from baseline to 8 weeks. Results are presented for twenty-two participants in the Cod group, twenty-two participants in the Salmon group and nineteen participants in the Control group, and are presented as medians and 25th, 75th percentiles. Changes within Cod group, Salmon group and Control group are compared using analysis of covariance (ANCOVA) with adjustment for baseline values after log transformation followed by Tukey's HSD Test. Bars with different letters are significantly different ($p < 0.05$).

Table 1: Participant characteristics at baseline

(Medians and 25th, 75th percentiles)

	Cod group (*n* 22)		Salmon group (*n* 22)		Control group (*n* 19)		*P*[*]
Men/Women	10/12		8/14		9/10		0.74
Age, years	47.2	38.0, 54.2	46.1	43.2, 52.6	40.1	31.0, 54.4	0.44
BMI, kg/m^2	31.0	29.2, 35.9	32.2	29.9, 34.7	33.9	29.1, 36.6	0.73
Body fat, %	39.3	28.3, 42.9	40.2	30.1, 43.0	39.3	33.1, 41.2	0.92
Muscle mass, %	34.4	32.5, 41.4	33.3	31.5, 40.0	33.9	32.2, 38.2	0.72

[*] Groups were compared at the baseline using the Pearson's χ^2 (categorical data) or the Kruskal–Wallis test (continuous data).

Table 2: Estimated daily dietary intake of vitamins based on 5 days dietary records at baseline and after 8 weeks*

(Medians and 25th, 75th percentiles)

	Baseline		8 weeks		$P^{\dagger}$	$P^{\ddagger}$	$P\S$
	Median	25th, 75th percentile	Median	25th, 75th percentile			
Vitamin A (retinol activity equivalents/day)							
Cod group	591	425,934	661	428,809	0.41	0.40	
Salmon group	634	517,941	618	553,734	0.30		
Control group	559	399,825	515	418,708	0.95		
Vitamin B1 (mg/day)							
Cod group	1.27	1.05,1.52	1.29	0.97,1.48	0.49	0.65	
Salmon group	1.36	1.09,1.68	1.32	1.04,1.46	0.72		
Control group	1.34	1.17,1.58	1.27	1.10,1.72	0.85		
Vitamin B2 (mg/day)							
Cod group	1.58	1.25,2.03	1.53	1.21,1.83	0.46	0.79	
Salmon group	1.50	1.33,1.85	1.37	1.27,1.69	0.21		
Control group	1.43	1.27,1.89	1.52	1.20,1.80	0.83		
Niacin (niacin equivalents/day)							
Cod group	37.8	28.2,44.8	34.3	23.5,40.8	0.15	0.56	
Salmon group	32.0	27.4,43.0	35.9	33.7,40.7	0.43		
Control group	34.3	29.5,39.3	33.3	28.7,47.3	0.42		
Vitamin B6 (mg/day)							
Cod group	1.53	1.24,1.83	1.46	1.16,1.62	0.10	0.59	
Salmon group	1.40	1.29,1.82	1.58	1.38,1.92	0.50		
Control group	1.42	1.23,1.75	1.61	1.22,1.84	0.66		
Folate (µg/day)							
Cod group	200	177,243	205	165,251	0.96	0.26	
Salmon group	214	172,244	194	158,218	0.18		
Control group	187	163,222	188	170,233	0.50		
Vitamin C (mg/day)							
Cod group	88.5	51.5,112.5	74.0	48.3,120.8	0.58	0.26	
Salmon group	71.0	46.0,92.8	59.0	36.0,95.5	0.77		
Control group	64.0	38.0,84.0	72.0	47.8,125.3	0.12		
Vitamin D (µg/day)							
Cod group	3.8	2.1,7.6	4.6	3.5,5.2	0.72	1.1×10^{-13}	0.22 A
Salmon group	4.7	2.9,6.7	11.9	10.0,14.0	2.1×10^{-4}		3.5×10^{-6} B
Control group	3.9	1.6,6.5	2.1	1.4,3.2	0.011		6.3×10^{-4} C
Vitamin E (alpha-tocopherol equivalents/day)							
Cod group	11.5	7.5,14.0	10.6	9.4,11.7	0.52	0.67	

Salmon group	11.5	6.9,14.3	11.1	8.6,12.7	0.69
Control group	10.0	7.2,12.7	10.6	8.4,14.5	0.34

*No differences were seen between the groups at the baseline (Kruskal Wallis test). Results are presented for twenty-two participants in the Cod group, twenty-two participants in the Salmon group and nineteen participants in the control group.

† Within-group changes are tested using the Wilcoxon's signed-ranks test.

‡ Changes within Cod group, Salmon group and Control group are compared using analysis of covariance (ANCOVA) with adjustment for baseline values after log transformation.

§ Changes within the Cod group are compared with the control group (A), changes within the Salmon group are compared with the control group (B), changes within the Cod group are compared with the Salmon group (C) using the Tukey's HSD Test when the ANCOVA test showed differences between the groups.

Table 3: Serum concentrations of vitamins at baseline and after 8 weeks*
(Medians and 25th, 75th percentiles)

	Baseline		8 weeks		*P*†	*P*‡	*P*§
	Median	25th, 75th percentile	Median	25th, 75th percentile			
All-trans retinol (vitamin A), µmol/l							
Cod group	2.26	1.92,2.64	2.30	1.86,2.59	0.53	0.97	
Salmon group	2.22	1,81,2.59	2.11	1.79,2.62	0.96		
Control group	2.06	1.66,2.41	2.05	1.63,2.44	0.76		
Thiamine (vitamin B1), nmol/l							
Cod group	6.5	5.7,7.9	6.3	5.6,7.0	0.18	0.19	
Salmon group	7.2	5.9,8.6	7.1	6.1,8.1	0.91		
Control group	7.1	6.6,8.2	7.3	6.2,9.1	0.92		
Thiamine monophosphate (vitamin B1), nmol/l							
Cod group	3.1	2.4,3.9	3.1	1.9,4.3	0.53	0.85	
Salmon group	3.6	2.7,5.0	3.8	3.2,4.7	0.83		
Control group	2.6	2.3,3.8	3.6	2.5,4.0	0.63		
Riboflavin (vitamin B2), nmol/L							
Cod group	16.2	10.1,24.0	17.6	8.0,21.9	0.45	0.88	
Salmon group	13.8	11.0,17.0	13.5	11.0,18.5	0.31		
Control group	15.8	13.2,23.0	16.1	9.3,27.2	0.78		
Flavine mononucleotide (vitamin B2), nmol/l							
Cod group	7.9	6.2,10.2	8.0	6.7,10.2	0.81	0.079	
Salmon group	9.3	6.3,13.5	8.4	5.3,11.6	0.012		
Control group	8.7	6.5,10.8	7.7	6.0,9.2	0.12		
Nicotinamide (vitamin B3), nmol/l							
Cod group	228	187,309	218	186,283	0.11	0.72	
Salmon group	200	120,291	207	168,256	0.77		
Control group	226	170,354	230	163,305	074		
N1-methylnicotinamide (vitamin B3), nmol/l							
Cod group	133	112,178	138	103,169	0.45	0.99	
Salmon group	115	73,166	130	82,160	0.47		
Control group	109	94,153	120	93,191	0.49		
Pyridoxal 5'-phosphate (vitamin B6), nmol/l							
Cod group	42.4	27.2,52.5	35.2	30.2,49.1	0.65	0.015	0.92[A]
Salmon group	38.9	27.1,52.5	46.5	31.3,65.0	0.017		0.30[B]
Control group	38.5	32.1,49.8	43.6	26.9,52.7	0.90		0.13[C]
Pyridoxal (vitamin B6), nmol/l							
Cod group	14.0	10.4,16.3	13.9	10.2,15.9	0.33	0.066	

Salmon group	12.7	11.5,18.4	16.1	13.2,18.4	0.038		
Control group	17.1	10.4,22.2	15.6	11.9,19.5	0.90		
4-Pyridoxic acid (vitamin B6), nmol/l							
Cod group	17.0	13.4,23.1	19.9	13.5,22.7	0.53	0.064	
Salmon group	18.2	14.5,22.8	22.1	17.3,27.2	0.016		
Control group	16.1	14.7,21.9	17.8	15.3,20.9	0.40		
Folate (vitamin B9), nmol/l							
Cod group	18.5	12.9,23.2	16.4	13.4,21.5	0.64	0.57	
Salmon group	19.4	13.4,25.6	17.3	12.5,20.2	0.036		
Control group	18.2	14.5,22.2	17.5	14.7,20.4	0.40		
Cobalamin (vitamin B12), pmol/l							
Cod group	336	262,399	339	264,393	0.82	0.094	
Salmon group	293	249,346	311	279,390	0.010		
Control group	282	272,379	301	239,365	0.84		
25-hydroxyvitamin D3, nmol/l							
Cod group	64.2	49.7,76.5	44.5	37.3,56.7	$4.0x10^{-5}$	0.0011	0.93^{A}
Salmon group	70.6	54.6,84.7	58.1	51.1,68.1	$8.0x10^{-5}$		0.0060^{B}
Control group	62.0	52.4,76.0	45.1	40.7,56.7	$8.0x10^{-4}$		0.013^{C}
Alpha-tocopherol (vitamin E), µmol/l							
Cod group	37.9	30.8,47.2	36.0	31.4,42.7	0.37	0.35	
Salmon group	31.7	29.0,36.2	32.0	27.9,37.5	0.31		
Control group	31.3	30.0,40.4	35.2	27.8,40.1	0.55		
Gamma-tocopherol (vitamin E), µmol/l							
Cod group	2.81	1.79,3.38	2.75	2.99,3.24	0.91	0.60	
Salmon group	2.06	1.83,3.23	2.17	1,73,2.98	0.22		
Control group	2.43	2.13,3.23	2.57	2.15,2.83	0.41		
Phylloquinone (vitamin K1), nmol/l							
Cod group	0.79	0.40,1.58	0.92	0.51,1.59	0.80	0.69	
Salmon group	0.54	0.30,1.21	0.64	0.47,1.04	0.37		
Control group	0.69	0.50,1.00	0.77	0.67,0.93	0.083		

*No differences were seen between the groups at the baseline (Kruskal Wallis test). Results are presented for twenty-two participants in the Cod group, twenty-two participants in the Salmon group and nineteen participants in the control group.

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