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Vitamin D status in a rural population of northern Norway with high fish liver consumption

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Abstract

Objective: To assess vitamin D status and the impact of three fish meals consisting of cod liver and fresh cod-liver oil on the plasma level of vitamin D metabolites in an area with high consumption of cod liver and cod-liver oil.

Design: Experimental field study.

Methods: Thirty-two volunteers from the Skjervøy (70°N) municipality in northern Norway were recruited to consume three traditional *mølje* meals, consisting of cod, cod liver, fresh cod-liver oil and hard roe, in one week. The liver and fresh cod-liver oil consumed by the participants were weighed and recorded. Blood samples were collected before the first meal, and subsequently 12 h and 4 days after the last meal. The blood samples were analysed for the vitamin D metabolites 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D ($1,25(OH)_2D$). All participants answered a semi-quantitative food-frequency questionnaire, which was used to estimate usual daily nutrient intake. The study was carried out in the last part of March 2001.

Results: The median daily vitamin D intake estimated from the questionnaire was 9.9 μg. The proportion of subjects with baseline 25(OH)D level below 50 nmol l^{-1} was 15.4% and none were below 37.5 nmol l^{-1}. Only '*mølje* consumption' and 'time spent in daylight' were significantly associated with baseline log 25(OH)D. The mean total intake of vitamin D in the three servings was 272 μg (standard deviation 94 μg), ranging from 142 to 434 μg. Relative to baseline plasma concentration, the mean level of 25(OH)D decreased slightly in both post-consumption samples ($P \leq 0.03$), while $1,25(OH)_2D$ peaked 12 h after the final meal ($P = 0.03$).

Conclusion: Three *mølje* meals provided, on average, an amount of vitamin D equal to 54 times the recommended daily dose. Subjects with food consumption habits that included frequent *mølje* meals during the winter sustained satisfactory vitamin D levels in their blood, in spite of the long 'vitamin D winter' (i.e. absence of ultraviolet-induced vitamin D production in the skin).

Keywords
Vitamin D status
Arctic diet
Cod liver
Cod-liver oil
Traditional food

Dietary sources of vitamin D are scarce. It is found mainly in fatty fish, cod-liver oil and fortified margarine or butter. Ultraviolet (UV)-induced skin production constitutes the main contributor to vitamin D in humans. UV radiation with wavelengths less than 320 nm is needed for this cutaneous photoconversion of provitamin D (7-dehydrocholesterol) to previtamin D, which is the precursor to vitamin D[1]. The great seasonal variation in UV levels has been found to cause seasonal fluctuations in the vitamin D status of humans[2]. Fish liver has traditionally been the most important vitamin D source for the coastal population of northern Norway during the winter months[3], since at these latitudes the sun-induced vitamin D production ceases for a considerable part of the year. The fish liver consumed was mainly from cod (*Gadus morhua* L.) and saithe (*Pollachius virens*), and the primary consumption followed the seasonal harvests. The cod liver season was linked to the spawning period and lasted about three months during the winter (most usually from January until March/April). Saithe liver was consumed from late summer until September/October.

A recent epidemiological investigation has confirmed the importance of diet to vitamin D status in the population of northern Norway[4]. Unpublished data from the nationwide Norwegian Women and Cancer Study (NOWAC)[5] have shown that there are still communities where fish liver and fresh fish-liver oil are consumed frequently. The coastal municipality of Skjervøy, which is situated at 70°N, was identified as one of those with the highest frequency of consumption. Fifty per cent of the participating subjects reported eating fish liver and fish-liver oil – or *mølje*, which is the Norwegian name of the

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dish – seven times per season or more. In a recent study among volunteers in Tromsø[6], the largest city in northern Norway, it was found that the average vitamin D consumption from one single fish meal containing fish liver and fresh cod-liver oil was 73.3 μg, which is about 15 times the recommended daily intake (5.0 μg)[7]. According to the unpublished NOWAC data, *mølje* consumption seems to be more common in the rural coastal northern areas than among the more urban centres.

Among the metabolites for determining the overall vitamin D status of an individual, 25-hydroxyvitamin D (25(OH)D) has been used most often[8]. Blood 25(OH)D concentration $\leq 37.5\,\text{nmol}\,\text{l}^{-1}$ has been designated as an indicator of moderate hypovitaminosis D[9,10] and concentrations $\geq 50\,\text{nmol}\,\text{l}^{-1}$ have been recommended[11,12]. The metabolite 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) is the biologically active form[1].

We recruited volunteers from the rural costal village of Skjervøy with the aim of assessing the impact of the traditional fish dish *mølje*, containing fish liver and fish-liver oil, on plasma levels of vitamin D and its metabolites in a population with high *mølje* consumption. We studied the effect of three consecutive fish meals on these parameters.

Methods and materials

Study subjects were recruited by announcement in the local newspaper. Inclusion criteria were either gender, above the age of 20 years and living in the municipality of Skjervøy. Thirty-two volunteers, 21 men and 11 women aged 38–61 years, wanted to take part in the study. The project was approved by the Regional Committee for Research Ethics, and all subjects signed a consent form.

The study schedule is outlined in Fig. 1. All participants answered the NOWAC food-frequency questionnaire (FFQ), which was used to estimate usual daily nutrient intakes. This questionnaire has been described in detail elsewhere[5,13]. A slight alteration of the questionnaire was made to allow discrimination between cod liver and saithe liver consumption and the recoding of usual consumption patterns. In addition we included questions on hours spent in daylight the previous week, as well as sun-seeking holidays and use of a solarium during the month prior to the study. The questionnaire also contained questions on gender, age, height and weight of the respondents.

The subjects were served three *mølje* meals with a 2-day interval during a 7-day period. All meals were served in the canteen at Skjervøy Upper Secondary School. The *mølje* meals were prepared in the traditional way by students attending the hotel and food-processing courses at this school. The fish was caught the day before or the same day it was served. Both the cod and the hard roe were boiled separately in water. The liver was divided and boiled in only small amounts of water and the oil derived from this constituted the fresh cod-liver oil. Participants served themselves and took as much as he or she wanted at the arranged fish buffet, but the amounts of liver and cod-liver oil on each participant's plate were weighed using digital scales and the weights recorded in grams by trained students at the school.

Blood samples were collected from participants before the first meal, and 12 h and 4 days after the last meal. The blood samples were collected into vacutainer tubes containing ethylenediaminetetraacetic acid (9 ml lavender top; Becton-Dickinson, Plymouth, UK). Participants' body weight and height were measured on day 8 or day 11. The study was carried out in the last part of March 2001. Participants were asked to maintain their usual diet during the study period.

Usual (i.e. self-reported) daily vitamin D intake was computed using information from the FFQ and the corresponding nutrient values reported in the Norwegian Food Composition Table[14], as well as our food analysis of liver and fresh cod-liver oil as described below. Owing to limited information on the nutrient content of different supplements, vitamin D contributions from dietary supplements other than cod-liver oil were not included in estimating the usual daily vitamin D intake for each participant.

Food sample analyses

Samples from the liver and fresh cod-liver oil servings were analysed for vitamin D according to the method described by Horvli and Lie[15]. In short, vitamin D was extracted using 96% ethanol/37.5% KOH (15:1 v/v), containing pyrogallol, ascorbic acid and an internal standard (vitamin D_2). Heating to 70°C for 20 min saponified the sample. After the addition of water, the sample was extracted twice with hexane using a whirl-mixer and a centrifuge. The combined hexane phases were extracted with water, and isopropanol was added before evaporation. The sample was cleaned up further

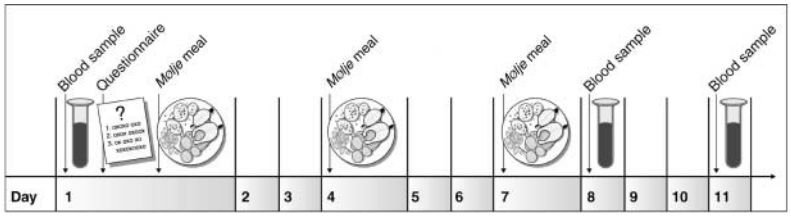

Fig. 1 Study design and blood sampling schedule (blood samples were collected before meal 1, and 12 h and 4 days after meal 3)

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using a high-performance liquid chromatography (HPLC) system consisting of a Spectra Physics P1000 isocratic pump, a Shimadzu SPD 6AV UV detector and a Shimadzu C-3A integrator, fitted with a Brownlee silica column (25 cm × 4.6 mm, 5 μm). For the analytical step, a C_{18} column (25 cm × 4.6 mm, 5 μm; Supelco Inc., Bellefonte, USA) was used.

For the preparative clean-up step, tetrahydrofuran–n-hexane (12.5:87.5 v/v) was used as the mobile phase. For the analytical step, chloroform–methanol–acetonitrile (6:12:82 v/v) was employed as eluent and the sample was dissolved in methanol. The flow rates were 1 ml min^{-1} for both columns. Vitamin D_2 (internal standard) and D_3 were detected on-line by the UV detector at 265 nm. These analyses were carried out by the Directorate of Fisheries, Institute of Nutrition, Bergen, Norway.

The vitamin D intake from the *mølje* meal was estimated on the basis of the vitamin D content of the liver and fresh cod-liver oil served, since the rest of the food items consumed (cod, potatoes and hard roe) contain little or no vitamin D.

Plasma levels of 25(OH)D and 1,25(OH)$_2$D

Blood plasma was collected and kept at −80°C until analysed for 25(OH)D and 1,25$(OH)_2$D according to modified versions of the methods described by Aksnes[16,17]. Briefly, 1.5 ml plasma samples were mixed with 2 ml of acetonitrile, vortexed and centrifuged at 1000 ***g*** for 10 min to remove proteins. The supernatants were collected, 3.5 ml of 0.1 M K_2HPO_4 (pH 10.5) was added, and the mixture applied to C-18-OH vacuum columns (Varian, USA). The columns were washed with 5 ml of distilled water, followed by a second wash with 5 ml of methanol–water (70:30 v/v), after which the 25(OH)D and 1,25$(OH)_2$D fractions were eluted with hexane–isopropanol (95:5 v/v) and evaporated to dryness with a gentle flow of nitrogen. The samples were dissolved in 250 μl of hexane–isopropanol–ethanol (95:2.5:2.5 v/v) and the collected fraction was separated on a silica column (Supelcosil, 15 cm × 4.6 mm, 3 μm; Supelco Inc.) by HPLC. The fractions containing 25(OH)D and 1,25$(OH)_2$D were collected, again evaporated to dryness with nitrogen gas, and then dissolved in ethanol. 25(OH)D was quantified by a radioreceptor assay (RRA) using human vitamin D binding protein from blood plasma as the binding protein, and 1,25$(OH)_2$D by an RRA using the 1,25$(OH)_2$D receptor from chick duodenal cytosol.

Serum parathyroid hormone

Serum parathyroid hormone (PTH) levels were measured with an Immulite analyser (Diagnostic Products, Los Angeles, CA, USA) on the basis of a two-site chemiluminescent immunometric assay. The Immulite analyser has a working range of 0.1–263 pmol l^{-1}.

Statistical analyses

Statistical analyses and nutrient calculations were done with the SAS software package, version 8.02 (SAS Institute, Cary, NC, USA). Log-transformed values for the variable 25(OH)D were used in the general linear models and *t*-tests because this variable was not normally distributed. When general linear models were chosen, a test for colinearity (variance of inflation factor) between the independent variables was applied. Independent variables were selected by backward elimination. For the analysis of baseline 25(OH)D levels, the subjects who reported that they had been on sun holiday or had recently used a solarium were excluded ($n = 4$) in addition to subjects for whom this information was missing ($n = 2$). Cod-liver oil supplement use was defined as taking it more than twice per week. Pearson correlation coefficients were calculated for the self-reported daily intakes of vitamin D and the 25(OH)D concentrations in blood at baseline. Paired sample *t*-tests and analysis of variance (ANOVA) with repeated measures design were used to compare changes in vitamin D metabolite levels with time.

Results

Some characteristics of the participants are shown in Table 1. The median self-reported usual vitamin D intake estimated from the semi-quantitative FFQ was 9.9 μg day^{-1}. According to the questionnaire data, 47% of the participants ate *mølje* two to three times per month or more, and 17% did so once per week or more during the *mølje* season (Fig. 2).

Table 1 Characteristics of the study sample ($n = 32$)*. Values are expressed as median or %

Characteristic	Men ($n = 21$)	Women ($n = 11$)	Total	Range
Age (years)	52	50	50	38–61
Body mass index (kg m^{-2})	29.4	28.3	29.2	19.8–35.6
Usual vitamin D intake (μg day^{-1}) (supplements other than cod-liver oil excluded)	11.5	5.9	9.9	3.0–40.1
Proportion of subjects reporting taking cod-liver oil supplements twice per week or more (%)	52.4	27.3	43.8	
Proportion of subjects who had been on a sun holiday or used a solarium the month prior to the study (%)	10.0	20.0	13.3	

* Subgroups may not total to 32 due to missing values.

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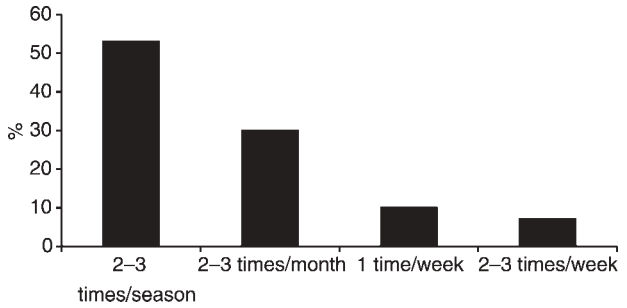

Fig. 2 Frequency of consumption during the *mølje* season (a season is estimated as about three months) among the study participants ($n = 30$)

The mølje *meals served*

The median intakes in grams of cod liver and fresh cod-liver oil consumed during the three meals are shown in Table 2, as well as the total percentage of fat and the amount of vitamin D per 100 g of food served per meal. The vitamin D content per 100 g of food varied two-fold between meals, while the fat content was approximately constant. As illustrated in Table 3, the total vitamin D intake from the served meals was higher only for men compared with women ($P = 0.004$) and not for any of the other parameters listed. The mean total quantity of vitamin D provided in the three meals was 271.9 μg (standard deviation (SD) 93.9 μg), ranging from 142.4 to 434.4 μg; the median intake was 264.1 μg.

Baseline 25(OH)D

The distribution pattern observed in the baseline concentrations of 25(OH)D is depicted in Fig. 3, corresponding to a mean concentration of 67.2 nmol l^{-1}. The proportion of subjects with 25(OH)D levels below 50 nmol l^{-1} was 15.4% and none were below 37.5 nmol l^{-1}. In the unadjusted analysis (Table 3), cod-liver oil supplement users had significantly higher 25(OH)D levels in their blood ($P = 0.04$) compared with non-users. Furthermore, subjects who spent <7 h per week in daylight the week prior to the study had significantly lower 25(OH)D concentrations in their blood compared with those who spent more time in daylight ($P = 0.02$). There was a significant trend ($P = 0.04$) in baseline 25(OH)D concentration by frequency of recalled *mølje* consumption. The proportion of subjects with baseline 25(OH)D levels below 50 nmol l^{-1} decreased with increased frequency of recalled *mølje* consumption.

The correlation coefficient for intake of vitamin D and levels in blood was $r = 0.30$, but not significant ($P = 0.13$). Subjects with a self-reported daily intake of vitamin D less than 10 μg had on average a 25(OH)D concentration of 62.4 (SD 13.4) nmol l^{-1} at baseline, compared with subjects having a daily intake of 10 μg or more, for whom it was 70.6 (SD 18.7) nmol l^{-1} (Table 3). However, this difference was not significant. In the multiple regression model only '*mølje* consumption' and 'time spent in daylight' were significantly associated with log 25(OH)D, adjusted for age and gender, while body mass index (BMI) had no effect as a predictor of vitamin D status at baseline (Table 4).

Table 2 Median total intake of cod liver and cod-liver oil from the three served *mølje* meals and the calculated nutrient content

Food item	Median intake (g)	Vitamin D (μg/100 g)	Total fat (%)
Cod liver			
Meal 1	108	50	48.6
Meal 2	115	40	50.9
Meal 3	95	80	44.7
Cod-liver oil			
Meal 1	28	90	96.2
Meal 2	25	60	96.4
Meal 3	25	120	93.8

Changes in vitamin D metabolites and PTH associated with mølje *meals*

There was a slight decrease in 25(OH)D levels at 12 h ($P = 0.03$) and 4 days ($P = 0.004$) after the last meal (Fig. 4). In the ANOVA for repeated measures design, there was a significant time effect for log 25(OH)D ($P = 0.001$) and for $1,25(OH)_2D$ ($P = 0.05$). Compared with baseline, there was an increase in $1,25(OH)_2D$ levels at 12 h ($P = 0.03$) after the third serving, but not after 4 days. The changes over time in 25(OH)D and $1,25(OH)_2D$ levels were not significantly associated with the total vitamin D consumed in the three served meals, nor with any of the following variables: gender, age, BMI, time spent in daylight, cod-liver oil supplement use, frequency of *mølje* consumption, and self-reported daily intake of vitamin D (ANOVA for repeated measurements, data not shown). The mean PTH serum concentrations (pmol l^{-1}) were 5.0 (SD 2.3), 5.4 (SD 2.9) and 3.8 (SD 1.7) at baseline, 12 h and 4 days after the last meal, respectively. The slight rise in PTH between baseline and 12 h after the last meal was non-significant, while the decrease at 4 days relative to baseline was highly significant ($P < 0.001$) when adjusting for age and sex.

Discussion

We found that the three consecutive *mølje* meals gave a short-term increase in plasma concentration of the active vitamin D metabolite $1,25(OH)_2D$ and a slight decrease in circulating 25(OH)D levels. However, baseline vitamin D status measured as 25(OH)D concentration was explained by both time spent in daylight the week before the study and frequency of self-reported *mølje* consumption. Furthermore, among these subjects from Skjervøy, 25(OH)D levels were relatively high and very few subjects had 25(OH)D below the recommended level.

In the beginning of April 2000, we conducted a similar study in the city of Tromsø, northern Norway (69°N), in which 33 volunteers were served a single *mølje* meal followed by repeated blood measurements of vitamin D[6]. Around 75% of these subjects had baseline concentrations

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Table 3 Plasma 25(OH)D levels at baseline and intake of vitamin D through the served *mølje* meals, by different characteristics*. Values are expressed as mean (SD) or %

Characteristic	Baseline 25(OH)D (nmol l^{-1})†‡	Proportion with baseline 25(OH)D level <50 nmol l^{-1} (%)†	Vitamin D consumed (μg) through the *mølje* meals‡
Total	67.2 (16.9)	15.4	272 (94)
Age (years)			
<50 ($n=14$)	66.8 (16.5)	21.4	284 (104)
≥ 50 ($n=18$)	67.2 (17.8)	11.1	262 (87)
	$P=0.92$		$P=0.52$
Gender			
Men ($n=21$)	68.6 (11.4)	9.5	305 (107)
Women ($n=11$)	63.9 (13.4)	27.3	209 (75)
	$P=0.53$		$P=0.004$
BMI (kg m^{-2})			
<25 ($n=9$)	67.4 (14.3)	0.0	239 (100)
≥ 25 ($n=23$)	67.1 (17.9)	20.0	285 (90)
	$P=0.97$		$P=0.22$
Cod-liver oil supplement use			
Yes ($n=14$)	73.8 (18.6)	7.1	260 (76)
No ($n=18$)	59.8 (11.3)	22.2	281 (107)
	$P=0.04$		$P=0.53$
Usual frequency of *mølje* consumption			
3 times per season or less ($n=16$)	60.7 (14.4)	25.0	262 (96)
2–3 times per month or more ($n=9$)	69.9 (15.1)	11.1	317 (102)
1–3 times per week ($n=5$)	77.7 (21.7)	0.0	263 (47)
	$P=0.04$§		$P=0.64$§
Time spent in daylight (h $week^{-1}$)			
<7 ($n=7$)	53.8 (10.6)	28.6	261 (110)
≥ 7 ($n=21$)	72.4 (16.1)	4.8	292 (89)
	$P=0.02$		$P=0.46$
Vitamin D intake (μg day^{-1})			
<10 ($n=16$)	62.4 (13.4)	18.8	245 (97)
≥ 10 ($n=16$)	70.6 (18.7)	12.5	299 (85)
	$P=0.23$		$P=0.11$

25(OH)D – 25-hydroxyvitamin D; SD – standard deviation; BMI – body mass index.
* All subgroups may not have $n=32$ due to missing values or exclusions.
† Subjects who reported that they had been on a sun holiday or used a solarium during the month prior to the study ($n=4$) were excluded.
‡ Statistical test: Student *t*-test for comparison of means.
§ Test for trend.

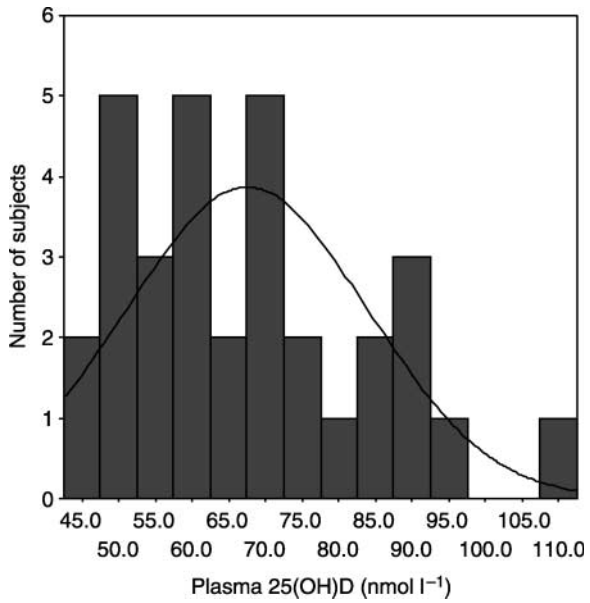

Fig. 3 Distribution of baseline plasma 25-hydroxyvitamin D (25(OH)D) concentrations among the study participants ($n=32$)

below the recommended level (50 nmol l^{-1}), and one quarter of the subjects were below the limit for moderate hypovitaminosis D (37.5 nmol l^{-1}). The relatively high concentrations found in the present study compared with the Tromsø group suggests that the regular consumption of traditional marine food plays an important role in vitamin D status.

The recommended daily dietary intake of vitamin D in the Nordic countries has been set at 5 μg[7]. The median self-reported vitamin D intake in the Skjervøy group was more than twice that found in nation-wide surveys conducted in Norway[5,18]. Moreover, it is known that significantly higher vitamin D intake occurs in northern Norway compared with populations in the more southern parts of the country[19]. By comparing the relative importance of UV-induced and dietary vitamin D on vitamin D status, a recent study has shown that diet is the major vitamin D source during winter until late spring for northern Norwegian populations[4]. The study in the late 1920s by Kloster[3] concluded that cod liver and cod-liver oil were the most important vitamin D sources for the costal population, and that rickets was common in places where

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Table 4 Multiple linear regression analysis identifying predictors for baseline log 25(OH)D ($n = 26$)*†

Variable	*t*-value	*P*-value
Cod-liver oil supplement use		
Yes ($n = 14$)	ref.	
No ($n = 18$)	1.47	0.16
Usual frequency of *mølje* consumption		
3 times per season or less ($n = 16$)	ref.	
2–3 times per month or more ($n = 9$)	1.06	0.30
1–3 times per week (n = 5)	2.14	0.05
Time spent in daylight (h week^{-1})		
< 7 ($n = 7$)	ref.	
≥ 7 ($n = 21$)	2.11	0.05
R^2_{adj}		0.51
P-value for model		0.04

25(OH)D – 25-hydroxyvitamin D; ref. – reference category.
* Subjects who reported that they had been on a sun holiday or used a solarium during the month prior to the study were excluded.
† Adjusted for age and gender.

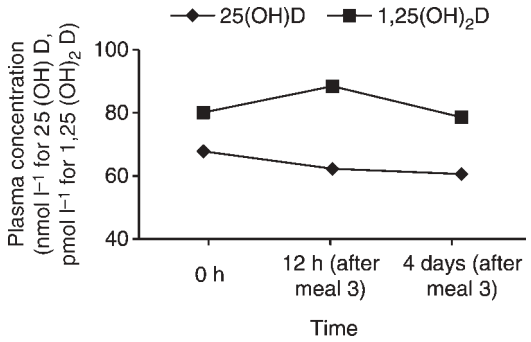

Fig. 4 Plasma concentrations of 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D (1,25(OH)$_2$D) by time among the study participants ($n = 32$)

there was limited access to fresh fish. Our results show that high consumption of *mølje* meals results in a satisfactory vitamin D status even when measured just after the end of the 'vitamin D winter'. This suggests that this traditional food compensates for the absence of sufficient sunlight-induced vitamin D production.

The finding that blood 25(OH)D level was not adequately explained by the estimate of total vitamin D intake, but by the frequency of *mølje* consumption, might be explained by a lower occurrence of misclassification on the *mølje* question as compared with the other dietary intake questions. Non-differential misclassification will dilute a true association. It has been shown previously, among subjects in northern Norway, that consumption of *mølje* is associated with vitamin D status[4]. In the above-mentioned study conducted in Tromsø[6], one meal of *mølje* increased the 25(OH)D level only for subjects with moderate hypovitaminosis D (25(OH)D ≤ 37.5 nmol l^{-1}). Thus, the relatively high baseline levels in the Skjervøy group could be one explanation for our finding that three *mølje* meals were not sufficient to raise the 25(OH)D concentration.

The increase in 1,25(OH)$_2$D could be attributed to the short-time increase in PTH, which activates hydroxylation of 25(OH)D in the kidneys[1]. The observed increase in PTH was, however, not significant. This inability to detect a change was most likely a consequence of low statistical power (small sample size). The observed increase in active vitamin D could also be due to the metabolite being bound to fat in circulation and thereby inactivated, thus causing a need to produce more 1,25(OH)$_2$D.

BMI has been shown to be associated with 25(OH)D levels in blood[6,20–22]. This was not found in our study, probably because of the narrow range in the BMI values, as most of the subjects were overweight. The results were, however, in accordance with the *mølje* study conducted in Tromsø[6], where there also was a short-term reduction in 25(OH)D after a *mølje* meal among the overweight subjects.

Lack of statistical power due to a small number of participants was a problem in the current study. Thus, substantial differences in 25(OH)D level such as those reported in Table 3 were found not to be significant. Neither was the study sample randomly selected, as the subjects were recruited based on their own willingness and initiative to participate. However, the participants' self-reported frequency of *mølje* consumption was in line with unpublished NOWAC data obtained in the municipality of Skjervøy and the recalled cod-liver oil supplement use in the present study was close to the prevalence found in nation-wide Norwegian[13] and northern Norwegian studies[4,22]. Since the study objective was to investigate the impact of *mølje* on vitamin D status in a high consumption group living in a rural area of northern Norway, the external validity of the findings is of less importance.

In conclusion, cod liver and fresh cod-liver oil can constitute a good vitamin D source. Three consecutive *mølje* meals on average provided 54 times the recommended daily dose of vitamin D. Subjects with food habits that include frequent *mølje* meals during the winter can sustain satisfactory vitamin D levels in their blood, despite the lack of UV-induced vitamin D production.

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