

CHANGE IN PLASMA LEVELS OF VITAMIN D AFTER CONSUMPTION OF COD-LIVER AND FRESH COD-LIVER OIL AS PART OF THE TRADITIONAL NORTH NORWEGIAN FISH DISH “MØLJE”.

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ABSTRACT

Objective. To assess changes in plasma 25-hydroxy vitamin D (25(OH)D) concentrations after ingestion of «Mølje», a traditional north Norwegian fish dish rich in vitamin D.

Methods. Thirty-three volunteers all living in the city of Tromsø, located in northern Norway (latitude 69°), were served a “Mølje“ meal consisting of cod, hard roe, cod liver, and fresh cod-liver oil. The amounts of liver, and cod-liver oil consumed were weighed and recorded. Blood samples were collected before the meal, and at 4 hours, 12 hours and 5 days after it. The cod liver and cod-liver oil were analysed for vitamin D content and the plasma samples for the metabolite 25(OH)D. Trends in plasma 25(OH)D levels during the five-day observation period were analysed. The study was conducted at the beginning of April of 2000.

Results. Among the 33 participating subjects, 69.7% had baseline plasma 25(OH)D concentrations below 50 nmol/l and for one-quarter of the subjects, they were < 37.5 nmol/l. The participants who acknowledged taking cod-liver oil supplements had significantly higher baseline 25(OH)D plasma levels at the outset of the study compared to those reporting not doing so (p=0.02). Changes in plasma 25(OH)D levels relative to baseline concentrations were significantly associated with the body mass index (p<0.01).

Conclusion. Vitamin D status in populations living in circumpolar areas needs more research to investigate to what degree people living in the Arctic areas are at increased risk for vitamin D insufficiency and to determine the role of the traditional diet in preventing such deficiency.

Keywords. 25-hydroxy vitamin D; cod liver; cod-liver oil; traditional diet; arctic diet

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INTRODUCTION

Vitamin D is essential for normal calcium and bone metabolism in humans (1) and is synthesized in the skin on exposure to sunlight (2). Ultra-violet B (UVB) radiation, with wavelengths between 280-320 nm, is needed for cutaneous vitamin D synthesis (2). Studies have shown that seasonal changes in the UVB radiation is responsible for the absence of cutaneous vitamin D production during winter and, not surprisingly, the length of the “vitamin D winter” increases with increased latitude (3,4).

Diet is the other source of vitamin D. Vitamin D is found in only a limited number of foods, such as fat fish, cod-liver oil, eggs, mushrooms, fortified milk, margarine, and butter.

In the population in north Norway, the impact of dietary vitamin D on vitamin D status is of importance, since, at this latitude (Tromsø city 69°N), no cutaneous vitamin D synthesis is likely to occur during a considerable part of the year.

The north Norwegian fish dish “Mølje”, consisting of cod, cod liver, hard roe and fresh cod-liver oil, has traditionally been an important part of the diet during the winter season among people living in the northern coastal areas of Norway. Cod liver and cod-liver oil are rich in vitamin D. According to the Norwegian Food Composition Table, 100 g of cod liver and 100 g of cod-liver oil contain 125 µg (5000 IU) and 261 µg (8640 IU) vitamin D, respectively. Cod muscle tissue contains only small amounts of vitamin D (1.5 µg/100 g). A dietary survey from northern Norway in 1931 did show that cod liver and fresh cod-liver oil were the most important vitamin D sources (5). According to that study, considerable amounts of both liver and oil were consumed and constituted one of the main fat sources. Today, the contribution of “Mølje” to the daily north Norwegian diet is not as pronounced as it was some time ago, but unpublished data from the nation-wide NOWAC-study (Norwegian Women and Cancer Study) (6) have shown that there are still areas where “Mølje” is consumed frequently.

The 25-hydroxy vitamin D (25(OH)D) concentration in blood has been considered the most valuable metabolite for determining the overall vitamin D status of an individual (1). A 25(OH)D concentration ≤ 37.5 nmol/l in blood has been used as an indicator of moderate hypovitaminosis D (7,8), while a 25(OH)D concentration ≥ 50 nmol/l has been considered as the recommended level (9,10). In

the present study, changes in plasma 25(OH)D were monitored after a single meal comprising cod, liver, and hard roe, corresponding to the traditional north Norwegian fish dish, “Mølje”.

MATERIAL AND METHODS

Study sample

Study subjects were recruited mainly from the Institute of Community Medicine, University of Tromsø. Thirty-three volunteers, 13 men and 20 women aged from 28 to 65 (average of 42), agreed to participate in the study. This project was approved by the Regional Committee for Research Ethics, and all subjects signed a consent form.

Study design and data collection

The participants were served the traditional north Norwegian fish dish “Mølje” with potatoes. The meal was prepared in the traditional way as follows: both the cod and the hard roe were boiled separately in water, while the liver was boiled in small amounts of water. The oil derived from this constituted the fresh cod-liver oil. Participants could eat as much as he, or she, wanted, but the amounts of liver and cod-liver oil consumed by each participant were weighed and recorded (Table III).

The study was carried out at the beginning of April of 2000. Its design and blood sample collection schedule are illustrated in Figure 1. The meal was served between 6 and 7 p.m. on day 1. Blood samples were collected just before the meal, and after 4 hours, 12 hours and 5 days. The ‘12-hour’ and ‘5-day’ samples were taken in the morning, before breakfast, while the ‘0-’ and ‘4-hour’ blood samples were taken

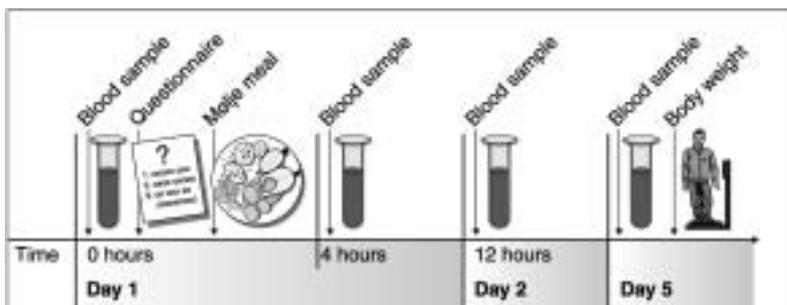


Figure 1. Study design and blood sampling schedule.

in the evening. The blood samples were collected employing EDTA-containing vacutainer tubes (BD Vacutainer Systems, Preanalytical Solutions Belliver Industrial Estate, Plymouth, UK).

Participants were asked to maintain their ordinary diet during the study period, but were instructed not to have any meals between lunch and the cod dish served on day 1. Body weight was measured on day 5.

To estimate the participants' usual daily vitamin D intake, all participants answered a semi-quantitative food-frequency questionnaire. The questionnaire explored the intake of fish and fish products rich in vitamin D, including questions on seasonal variations of consumption for each fish variety. Use of vitamin D-fortified foods, such as margarine, butter and milk were also addressed, as well as the consumption of cod-liver oil supplements, including seasonal variations and the use of other fish oil capsules and vitamin supplements. Usual daily vitamin D intake was computed using the vitamin figures from nutrient values reported in the Norwegian Food Composition Table (11), the specified content of supplements taken, and our food analysis of liver and fresh cod-liver oil as described below. The questionnaire also contained questions on the gender, age, height and weight of the respondents.

Food sample analyses

Samples from the served liver and fresh cod-liver oil were analysed for vitamin D, according to the method described by Horvli and Lie (12). In short, vitamin D was extracted using 96 % ethanol/ 60 % KOH, containing pyrogallol, ascorbic acid and an internal standard (vitamin D₂). Heating to 70°C for 20 minutes saponified the sample. After addition of water, the sample was extracted twice with hexane using a whirl-mixer and centrifuge. The combined hexane phases were extracted with water, and iso-propanol was added before evaporation. The sample was further cleaned up using a HPLC system consisting of a Spectra Physics P1000 isocratic pump, a Shimadzu SPD 6AV UV-detector, a Shimadzu C-3A integrator, fitted with a Brownlee silica column (25 cm x 4.6 mm, 5 µm). For the analytical step, a C₁₈ column (25 cm x 4.6 mm, 5 µm Supelco Inc., Bellefonte, USA) was used.

For the preparative clean-up step, tetrahydrofurane : n-hexane (in the ratio 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 for both columns. Vitamin D₂ (internal standard) and D₃ were detected on-line by the UV-detector at 265 nm. The food analysis was performed by the Directorate of Fisheries, Institute of Nutrition, Bergen, Norway.

The vitamin D intake from the “Mølje” meal was estimated based on the vitamin D content of the liver and the 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

The blood samples were analysed for 25(OH)D. Blood plasma was collected and stored at -80°C until analysis, which was performed by a modified version of the method described by Aksnes (13,14). Briefly, 0.1 ml plasma samples were mixed with 1.4 ml 0.15 M NaCl. To estimate the 25(OH)D recovery, [³H]25(OH)D₃ (1500 DPM) in 50 µl ethanol was added. The plasma dilutions were mixed with 2 ml acetonitrile, vortexed and centrifuged at 1,000 x g for 10 min to remove proteins. The supernatants were collected, 3.5 ml 0.1 M K₂HPO₄ (pH 10.5) were added, and the mixture was applied to C-18-OH vacuum columns (Varian, USA). The columns were washed with 5 ml distilled H₂O, followed by a second wash with 5 ml methanol: H₂O (70:30, v/v), after which the 25 (OH)D₃ fractions were eluted with hexane:isopropanol (95:5, v/v), and evaporated with nitrogen gas. The samples were dissolved in 250 µl hexane: isopropanol: ethanol (95:2.5:2.5, v/v) and separated on a Supelcosil silica column (15 cm x 4.6 mm, 3 µm; Supelco Inc, Bellefonte, USA) by HPLC. The fractions containing 25(OH)D were evaporated with nitrogen gas and dissolved in 400 µl ethanol. 25(OH)D was quantified by a radio-receptor assay (RRA) using human vitamin D-binding protein from blood plasma as the binding protein. The inter-assay coefficient of variation in this method was 8.5 % (estimated based on ten measurements of the same blood sample on ten different days).

Statistical Analyses

Statistical analyses and nutrient calculations were performed employing the SAS software package, version 6.12 (SAS Institute, 1996). To assess the relation between the daily vitamin D intake estimated from the questionnaire data and 25(OH)D levels in blood, the Pearson's correlation coefficient was calculated. When comparing both plasma 25(OH)D mean values and mean plasma 25(OH)D changes at 5 days

as a function of age, body mass index (BMI = weight/height²), gender and supplement use, the Student t-test was used. The Mann-Whitney test was used for analysing differences in vitamin D consumed through the “Mølje” meal by these characteristics. Changes in plasma 25(OH)D levels over time were assessed by ANOVA with repeated measurement design. When considering the amounts consumed as predictors for the post-meal 25(OH)D levels (difference between baseline and 5-day levels), a multivariate regression model was used.

RESULTS

Some characteristics of the participating subjects are shown in Table I. The mean daily vitamin D intake for the whole group, as estimated from the questionnaire, was 7.9 µg/day (95% C.I.; 5.8, 10.0), when vitamin D contributions from supplements other than cod-liver oil supplements were excluded. Six out of the 10 subjects who reported using a cod-liver oil supplement more than twice per week, did so in addition to other vitamin supplements. The mean value at baseline for plasma 25(OH)D was 51.2 nmol/l (SD=24.0). Among the participating subjects, 69.7% had baseline plasma 25(OH)D concentrations below the recommended level of 50 nmol/l. One-quarter of the subjects had levels below 37.5 nmol/l, which has been set as a limit for moderate hypovitaminosis D.

The distribution of the plasma 25(OH)D levels at baseline is shown in Figure 2. The correlation between estimated daily vitamin D intake, based on the questionnaire and 25(OH)D levels in blood, was $r=0.38$ ($p=0.03$). The participants who reported taking a cod-liver oil supplement more than twice per week had significantly higher

Table I. Characteristics of the study sample (n=33).

Characteristics	Mean or %	SD	Range
Mean age	42	8.0	28-65
BMI ^a	24.2	2.9	19.5-29.4
Usual vitamin D intake (µg/day) (supplements other than cod-liver oil excluded)	7.9	5.9	1.3-27.0
Usual vitamin D intake (µg/day) (supplements included)	8.8	6.3	1.3-27.0
Sex, % women	60.6		
Proportion of subjects reporting taking cod liver oil supplement twice per week or more (%)	30.3		

^a (weight / height²)

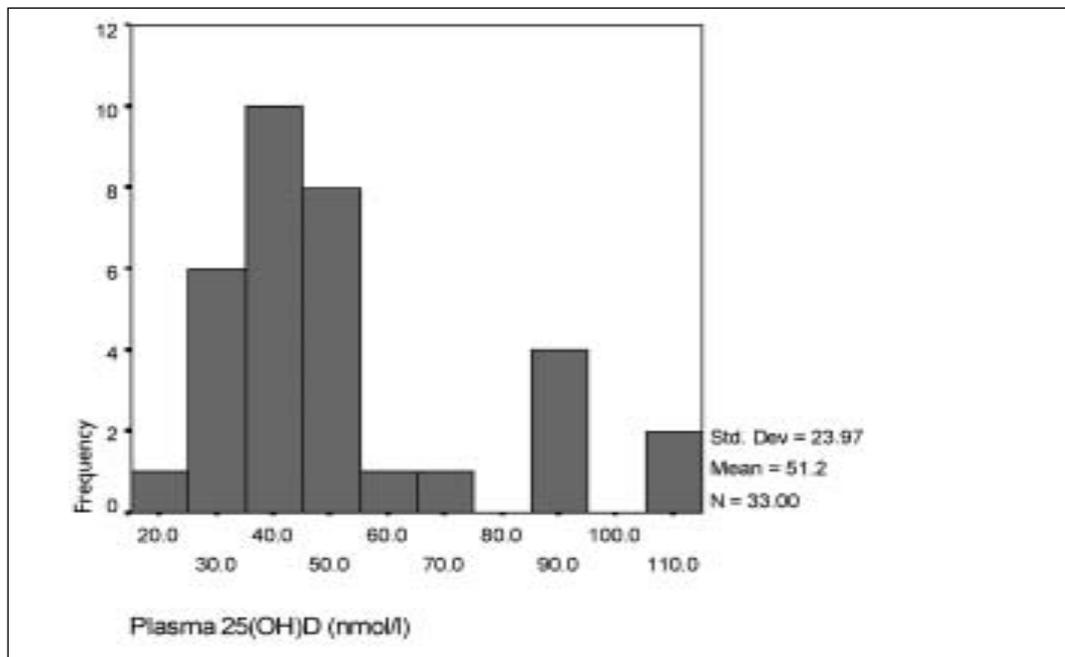


Figure 2. The distribution of 25(OH)D at baseline.

25(OH)D plasma levels at baseline compared to those reporting not using, or taking cod-liver oil twice per week or less ($p=0.02$), with mean values of 66.3 nmol/l (SD=25.7) and 44.7 nmol/l (SD=20.4), respectively. None of the subjects reporting not using, or consum-

Table II. Vitamin consumed through the “Mølje-meal” and plasma 25(OH)D levels by different characteristics.

Characteristic	Baseline 25(OH)D ^a Mean (SD)	% below 50 nmol/l	Mean vitamin D consumed (mg) through the “Mølje-meal” ^b	Mean plasma 25(OH)D change at 5-days (SD) ^a
Age				
< 42 (n=15)	47.5 (23.5)	80	59.6 (35.5)	-0.1 (5.0)
≥ 42 (n=18)	54.4 (24.6)	61	84.7 (63.9)	1.3 (7.2)
	n.s		n.s	n.s
BMI				
< 25 (n=23)	50.7 (24.8)	74	72.5 (58.2)	2.4 (5.9)
≥ 25 (n=10)	52.4 (23.3)	60	75.1 (44.2)	-3.3 (5.4)
	n.s		n.s	$p=0.02$
Gender				
Men (n=13)	57.1 (29.6)	45	67.9 (40.9)	-0.8 (6.6)
Women (n=20)	47.4 (19.4)	75	76.8 (61.3)	1.6 (5.9)
	n.s		n.s	n.s
Supplements				
Yes (n=10)	66.3 (25.7)	40	100.4 (75.0)	1.8 (6.3)
No (n=23)	44.7 (20.4)	83	61.5 (37.5)	0.1 (6.3)
	$p=0.02$		n.s	n.s

^aStudent t-test ^b Man-Whitney test

Table III. Mean intake of liver, fresh cod-liver oil, and vitamin D calculated from the served “Mølje-meal”.

	Weight (g)	SD	Range (g)	Vit D ₃ (μ g)	SD	Range (μ g)	Vit D content/100 g boiled food item (μ g)
Liver	113.6	50.5	33-281	50.0	22.2	14.5-123.6	44
Fresh cod-liver oil	20.8	31.6	0-152	23.3	35.4	0-170.2	122

ing, cod-liver oil twice per week or less, were taking other vitamin supplements more than twice per week. Baseline 25(OH)D plasma levels increased with supplement use, while gender, BMI and age had no significant effect (Table II). The mean plasma 25(OH)D change at 5 days was significantly different between BMI groups ($p=0.02$). Supplement users seemed to consume more vitamin D through the “Mølje” meal compared to the non-supplements users, but this difference was not significant.

The vitamin D content per 100 g cooked food item was nearly three times higher for cod-liver oil than for the liver (Table III). The amount consumed in the served meal of both liver and cod-liver oil varied considerably among the subjects, ranging from 33 to 281 g and from 0 to 152 g for the liver and the cod-liver oil, respectively. The calculated mean total vitamin D intake from the served “Mølje” meal was 73.3 μ g, ranging from 14.5 to 293.8 μ g.

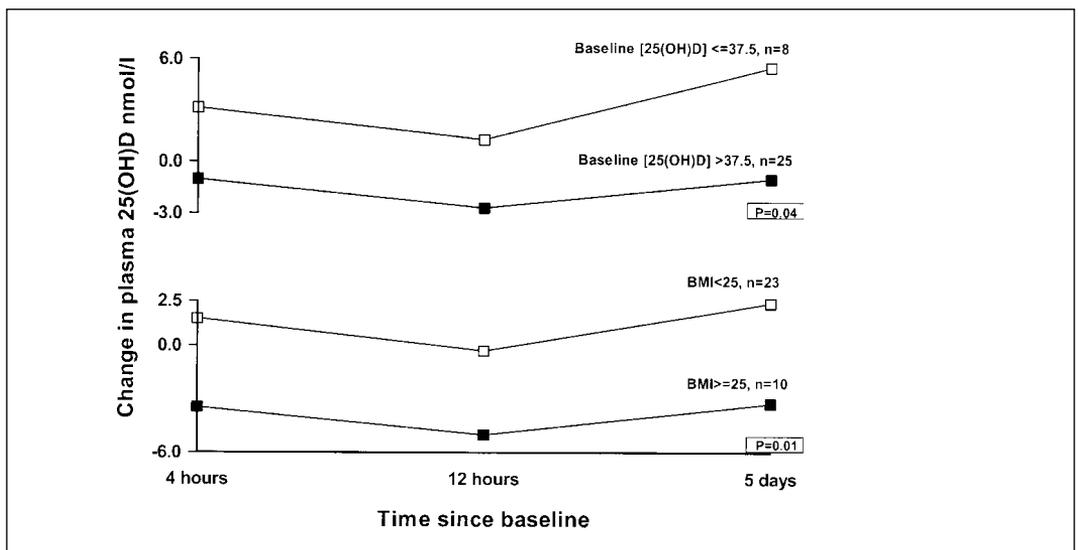


Figure 3. Relative change from baseline in 25(OH)D by BMI group or 25(OH)D baseline levels after consumption of a “Mølje” meal.

The mean plasma 25(OH)D levels after consumption of “Mølje” were 51.2 nmol/l (SD=23.2), 49.5 nmol/l (SD=23.5), and 52.2 nmol/l (SD=24.0) after 4 hours, 12 hours and 5 days, respectively. Ingested vitamin D from the meal did not predict variation in plasma 25(OH)D over time ($p=0.86$). When the response variable was alteration in plasma levels of 25(OH)D relative to baseline levels, and baseline concentrations was re-coded into a dichotomous variable ($[25(OH)D] < 37.5$ nmol/l and $[25(OH)D] \geq 37.5$ nmol/l), the plasma level change was significantly different between the two groups ($p=0.02$). As shown in Figure 3, the group with moderate deficiency had, on average, an increase, while subjects with 25(OH)D concentrations above 37.5 nmol/l showed a decrease. Furthermore, change in plasma level relative to baseline was significantly associated with BMI ($p < 0.01$) (Figure 3). Subjects with a BMI < 25 kg/m² showed a mean increase in 25(OH)D levels at 4 hours and 5 days; by contrast, subjects with BMI ≥ 25 kg/m² exhibited mean decreases at 4 hours, 12 hours and 5 days. The observed difference in plasma 25(OH)D change between the two BMI groups did not depend on time (i.e., non-significant interaction term for BMI group and time). The amount consumed did not predict the post-meal levels in plasma 25(OH)D (calculated as the difference between the 5-day and baseline levels) when adjusting for 25(OH)D baseline levels (low vs. high) and BMI in the multivariate regression model ($p=0.17$).

DISCUSSION

The main finding in this study was a high prevalence of 25(OH)D levels in blood below recommended levels, especially among those not taking supplements. Further, the BMI and baseline plasma 25(OH)D concentrations determined the change in mean 25(OH)D levels in the blood after consumption of a single fish meal with cod liver and cod-liver oil as major components.

The vitamin D concentrations found in the present study were low for the majority of the participants and, thus, contradict the anecdotal assumption that vitamin D status in north Norway is satisfactory (5). Since sunshine exposure has been considered to be the main source of vitamin D, its status is known to drop during winter and improve during summer months (16-19). Lehtonen-Veromaa *et al* (7) found a prevalence of 25(OH)D levels ≤ 37.5 nmol/L in excess

of sixty percent during winter, which reduced to less than two percent during the summer among 15-year-old Finnish girls in Turku, Southwest Finland. Vitamin D production in skin has been associated not only with the season, but also with latitude (2). Webb *et al* (3) found that, in Boston, USA (42°N), vitamin D production was curtailed from November to February, while in Edmonton, Canada (52°N), the photosynthesis of vitamin D ceased in October and did not occur again until April. This seasonal-latitude dependency of vitamin D production in the skin suggests that, in Tromsø, which is located at 69° north, the sun's role as a vitamin D source is limited to a few months of the year. Our study was carried out during the last part of the "vitamin D winter" (April). Thus, one would expect to observe the lowest vitamin D levels at this time of the year and our findings would, therefore, not necessarily constitute a valid measure of the yearly mean value. However, UVB radiation levels are also affected by the weather, and there is most likely a great variation in the contribution of the north Norwegian summers to vitamin D production from one year to another. The assumption that vitamin D status in north Norway is adequate, due to sufficient dietary intake and sufficient light during the summer months, is primarily based on one study conducted by Vik *et al* (15) in 1977/1978. In the latter study, blood samples were collected every two months throughout a year from seventeen laboratory workers at the University Hospital of Tromsø. The blood samples were analysed for 25(OH)D content and for several other characteristics as well. Subjects taking supplements were not included. The mean values for 25(OH)D for March (no data for April) found by Vik *et al* (15) did not differ significantly from those found in our study, when excluding the individuals reporting the use of supplements (mean=51.5 nmol/l, SD=19.5 v.s. mean=44.7, SD=20.4, $p=0.30$). However, Vieth and Carter (20) have warned against presenting only mean \pm S.D. values when assessing changes in 25(OH)D levels. They suggested a statistic that highlights the prevalence of insufficiency. About 50 % of the participants in Vik and colleagues' study had values below 50 nmol/l. Based on their distribution pattern of serum 25(OH)D, it is estimated that nearly 40 % of the subjects had values below 37.5 nmol/l, with two subjects having values less than 20 nmol/l; the latter concentration defines severe hypovitaminosis D (8). The Vik *et al* (15) survey is commonly referred to, despite this high prevalence of insufficient vitamin D status and the small sample size. It has been used as an argument for the

of an adequate vitamin D status in the northern Norwegian population. Their conclusion might have contributed to a neglect of potential nutritional and related health problems. To our knowledge, there has been no randomised population-based survey on 25(OH)D levels in north Norway. Although the participants in the present study constituted a select group (mainly academics), the results, when considered along with the data from the study by Vik and colleagues, suggest that the population in the North is at increased risk of vitamin D deficiency, especially during winter.

We found significantly higher levels of 25(OH)D in subjects using supplements compared to non-supplement users. The association between the use of supplements and the 25(OH)D blood concentration has been reported in several studies (8,21). Cod-liver oil was the most frequently used supplement. The use of cod-liver oil in Norway has been estimated to be 36.8 % and 33.9% for men and women, respectively (22). These numbers are in agreement with the present study, as 10 out of 33 participants used cod-liver oil supplements more than twice per week.

The correlation between the estimated daily vitamin D intake and 25(OH)D blood concentrations has been shown to be significant among subjects with low sun exposure (23,24). In our study, no such data was collected. However, the significant correlation between estimated daily intake and 25(OH)D blood concentrations supports the hypothesis that diet is the main contributor to vitamin D levels among people living at northern latitudes during winter. Our work emphasises the necessity of a sufficient dietary intake of vitamin D in circumpolar areas.

We found the change in 25(OH)D levels after ingestion of the “Mølje” meal to be associated with the BMI. An inverse association between the BMI and 25(OH)D in blood has been reported in other studies (16,25-27). Obesity has been considered to be an independent predictor of vitamin D deficiency (28). The reason for an increased risk of vitamin D deficiency in obese individuals remains unknown. However, it has been postulated by Bell *et al* (29) that obesity modifies the vitamin D endocrine pathways; this is attributed to a feedback inhibition by increased serum levels of the active metabolite 1,25(OH)₂D produced during the hepatic synthesis of 25(OH)D. It has also been suggested that the metabolic clearance of vitamin D may increase in obesity, possibly through an enhanced uptake by

adipose tissue (30) related to the larger fat mass and the consequently larger pool size in obese individuals (16). In our study, the only vitamin D metabolite measured was 25(OH)D. Thus, a reduction in 25(OH)D caused by an increase in active vitamin D could not be assessed. However, the results from our work indicate that the BMI predicts the metabolic response to a meal rich in vitamin D.

The ingested dose of vitamin D from one “Mølje” meal was not sufficient to raise the 25(OH)D level for the group. The stability of the mean 25(OH)D concentrations could also be due to the low baseline 25(OH)D values. The 25(OH)D metabolite has been considered a marker of medium-to-long-term vitamin D availability. Bates *et al* (31) have suggested that when vitamin D supplements are given to individuals with vitamin D deficiency, 1,25(OH)₂D will rise rapidly to normal values, whereas 25(OH)D will remain low until a reserve accumulates. This was, however, not supported by our data showing that individuals with moderate vitamin D deficiency at baseline exhibited a relative increase in plasma 25(OH)D. This must be interpreted with caution, as this analysis is based on a small number of subjects (n=8). Wortsman *et al* (27) found that, after ingestion of 1.25 mg vitamin D, 25(OH)D rose rapidly until approximately 10 hours after intake, declining slightly thereafter. According to this, one would expect the blood collected after 12 hours to have levels closer to the peak level. However, in our study, the mean 25(OH)D levels measured at 12 hours were the lowest for both normal and overweight subjects. The mean ingested dose of vitamin D from the “Mølje” meal was less than six percent of the dose in the study by Wortsman *et al* (27), which suggests that one “Mølje” meal was not sufficient to generate a peak level of plasma 25(OH)D for the group.

In conclusion, one meal was not sufficient to change the overall vitamin D status of the study group, but repeated meals over time may be anticipated to have a beneficial effect. However, modest beneficial effects were noted for those with low baseline levels and for leaner individuals. The vitamin D status and related health outcomes in populations living in circumpolar areas require more research to investigate to what degree people are at increased risk for vitamin D insufficiency during the winter, and to elucidate further the role of the traditional diet in preventing deficiency. Emphasis should also be on the intensity of different wavelengths within the UVB spectra at high latitudes and its impact on vitamin D status.

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REFERENCES

1. Holick MF. The use and interpretation of assays for vitamin D and its metabolites. *J Nutr* 1990; 120 Suppl 11: 1464-1469.
2. Webb AR, Holick MF. The role of sunlight in the cutaneous production of vitamin D3. *Annu Rev Nutr* 1988; 8: 375-399.
3. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: Exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* 1988; 67: 373-378.
4. Chapuy MC, Preziosi P, Maamer M et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997; 7: 439-443.
5. Kloster J. The distribution and frequency of rickets in one of the fishery districts of Finnmark and relation of diet to the disorder. *Acta Pædiatrica* (1931) 12, 1-82.
6. Hjaråker A, Engeset D, Brustad M, Lund E. Fiskekonsum og kreftrisiko blant norske kvinner- The Norwegian women and cancer study (NOWAC). *Nor J Epidemiol* 2000; 10: 63-70.
7. Lehtonen-Veromaa M, Mottonen T, Irjala K et al. Vitamin D intake is low and hypovitaminosis D common in healthy 9- to 15-year-old Finnish girls. *Eur J Clin Nutr* 1999; 53: 746-751.
8. Thomas MK, Lloyd JD, Thadhani RI et al. Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998; 338: 777-783.
9. Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998; 351; 805-806.
10. Schmidt-Gayk H, Bouillon R, Roth HJ. Measurement of vitamin D and its metabolites (calcidiol and calcitriol) and their clinical significance. *Scand J Clin Lab Invest Suppl* 1997; 227: 35-45.
11. National Nutrition Council & Norwegian Food Control Authority. The norwegian food composition Table 1995. Oslo: Universitetsforlaget.
12. Horvli O and Lie Ø. Determination of vitamin D3 by HPLC. *Fisk Dir Ser Ernæring* 1994; 6: 163-75.
13. Aksnes L. Quantitation of the main metabolites of vitamin D in a single serum sample. I. Extraction, separation and purification of metabolites. *Clin Chim Acta* 1980; 104: 133-146.
14. Aksnes L. Quantitation of the main metabolites of vitamin D in a single serum sample. II. Determination by UV-absorption and competitive protein binding assays. *Clin Chim Acta* 1980; 104: 147-159.
15. Vik T, Try K, Stromme JH. The vitamin D status of man at 70 degrees north. *Scand J Clin Lab Invest* 1980; 40: 227-232.
16. Need AG, Morris HA, Horowitz M, Nordin C. Effects of skin thickness, age, body fat, and sunlight on serum 25-hydroxyvitamin D. *Am J Clin Nutr* 1993; 58: 882-885.
17. Harris SS, Dawson-Hughes B. Seasonal changes in plasma 25-hydroxyvitamin D concentrations of young American black and white women. *Am J Clin Nutr* 1998; 67: 1232-1236.
18. Holick MF. Environmental factors that influence the cutaneous production of vitamin D. *Am J Clin Nutr* 1995; 61(3 suppl): 638S-645S.

19. Vieth R, Cole DE, Hawker GA, Trang HM, Rubin LA. Wintertime vitamin D insufficiency is common in young Canadian women, and their vitamin D intake does not prevent it. *Eur J Clin Nutr* 2001; 55: 1091-1097.
20. Vieth R, Carter G. Difficulties with vitamin D nutrition research: objective targets of adequacy, and assays for 25-hydroxyvitamin D. *Eur J Clin Nutr* 2001; 55: 221-222.
21. Davies PS, Bates CJ, Cole TJ, Prentice A, Clarke PC. Vitamin D: seasonal and regional differences in pre-school children in Great Britain. *Eur J Clin Nutr* 1999; 53: 195-198.
22. Johansson L, Solvoll K, Bjørneboe G-EA, Drevon CA. Dietary habits among Norwegian men and women. *Scand J Nutr/Näringsforskning* 1997; 41: 63-70.
23. Lips P, van Ginkel FC, Jongen MJ, Rubertus F, van der Vijgh WJ, Netelenbos JC. Determinants of vitamin D status in patients with hip fracture and in elderly control subjects. *Am J Clin Nutr* 1987; 46: 1005-1010.
24. Salamone LM, Dallal GE, Zantos D, Makrauer F, Dawson HB. Contributions of vitamin D intake and seasonal sunlight exposure to plasma 25-hydroxyvitamin D concentration in elderly women. *Am J Clin Nutr* 1994; 59: 80-86.
25. Jacques PF, Felson DT, Tucker KL et al. Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. *Am J Clin Nutr* 1997; 66: 929-36.
26. Lind L, Hanni A, Lithell H, Hvarfner A, Sorensen OH, Ljunghall S. Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. *Am J Hypertens* 1995; 8: 894-901.
27. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; 72: 690-693.
28. Semba RD, Garrett E, Johnson BA, Guralnik JM, Fried LP. Vitamin D deficiency among older women with and without disability. *Am J Clin Nutr* 2000 72, 1529-1534.
29. Bell NH, Epstein S, Greene A, Shary J, Oexmann MJ, Shaw S. Evidence for alteration of the vitamin D-endocrine system in obese subjects. *J Clin Invest* 1985; 76: 370-373.
30. Liel Y, Ulmer E, Shary J, Hollis BW, Bell NH. Low circulating vitamin D in obesity. *Calcif Tissue Int* 1988; 43: 199-201.
31. Bates CJ, Thurnham DI, Bingham SA, Margetts BM, Nelson M. Biochemical markers of nutrient intake. Margetts, B. M. and Nelson, M. *Design Concepts in Nutritional Epidemiology*. 2 ed. London: Oxford 1998; 170-240.

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