Prevention of covid-19 and other acute respiratory infections with cod liver oil supplementation, a low dose vitamin D supplement: quadruple blinded, randomised placebo controlled trial

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ABSTRACT

OBJECTIVE
To determine if daily supplementation with cod liver oil, a low dose vitamin D supplement, in winter, prevents SARS-CoV-2 infection, serious covid-19, or other acute respiratory infections in adults in Norway.

DESIGN
Quadruple blinded, randomised placebo controlled trial.

SETTING
Norway, 10 November 2020 to 2 June 2021.

PARTICIPANTS
34 601 adults (aged 18-75 years), not taking daily vitamin D supplements.

INTERVENTION
5 mL/day of cod liver oil (10 µg of vitamin D, n=17 278) or placebo (n=17 323) for up to six months.

MAIN OUTCOME MEASURES
Four co-primary endpoints were predefined: the first was a positive SARS-CoV-2 test result determined by reverse transcriptase-quantitative polymerase chain reaction and the second was serious covid-19, defined as self-reported dyspnoea, admission to hospital, or death. Other acute respiratory infections were indicated by the third and fourth co-primary endpoints: a negative SARS-CoV-2 test result and self-reported symptoms. Side effects related to the supplementation were self-reported. The fallback method was used to handle multiple comparisons.

RESULTS
Supplementation with cod liver oil was not associated with a reduced risk of any of the co-primary endpoints. Participants took the supplement (cod liver oil or placebo) for a median of 164 days, and 227 (1.31%) participants in the cod liver oil group and 228 (1.32%) participants in the placebo group had a positive SARS-CoV-2 test result (relative risk 1.00, multiple comparison adjusted confidence interval 0.82 to 1.22). Serious covid-19 was identified in 121 (0.70%) participants in the cod liver oil group and in 101 (0.58%) participants in the placebo group (1.20, 0.87 to 1.65). 8546 (49.46%) and 8565 (49.44%) participants in the cod liver oil and placebo groups, respectively, had ≥1 negative SARS-CoV-2 test results (1.00, 0.97 to 1.04). 3964 (22.94%) and 3834 (22.13%) participants in the cod liver oil and placebo groups, respectively, reported ≥1 acute respiratory infections (1.04, 0.97 to 1.11). Only low grade side effects were reported in the cod liver oil and placebo groups.

CONCLUSION
Supplementation with cod liver oil in the winter did not reduce the incidence of SARS-CoV-2 infection, serious covid-19, or other acute respiratory infections compared with placebo.

TRIAL REGISTRATION
ClinicalTrials.gov NCT04609423.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Vitamin D has been suggested as having a role in the prevention of covid-19, but most studies have been observational

A recent meta-analysis of 46 randomised controlled trials showed that vitamin D supplementation decreased the risk of acute respiratory infections compared with placebo, but the effect was small

WHAT THIS STUDY ADDS

Of 34 601 unselected adult participants, no difference in the incidence of SARS-CoV-2 infection, serious covid-19, or acute respiratory infections was found for those randomised to daily supplements of low dose vitamin D (cod liver oil) or placebo (corn oil) during the winter

The cod liver oil and placebo group had similar side effects, and only low grade side effects were reported

Introduction

Vitamin D has received much attention during the covid-19 pandemic for its potential role in preventing and treating covid-19.[1-9] Preclinical studies have reported a role for vitamin D metabolites in the immune responses to respiratory viruses, although the mechanisms are not fully understood.[10] Low levels of 25-hydroxyvitamin D_3 ($25(OH)D_3$) have been associated with an increased risk of acute respiratory infections.[11] A recent meta-analysis, examining 46 randomised controlled trials, concluded that vitamin D supplementation (400-1000 IU/day or 10-25 µg/day) decreased the risk of acute respiratory infections compared with placebo.[12]

Serious covid-19 has been associated with increased inflammation with uncontrolled activation of immune cells and excessive release of proinflammatory cytokines.[13] Long chained omega 3 fatty acids,

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particularly eicosapentaenoic acid and docosahexaenoic acid, have been reported to have anti-inflammatory effects.[14-17] Ensuring adequate levels of these fatty acids and vitamin D has been proposed as a cost effective measure to prevent SARS-CoV-2 infection and serious covid-19.[13]

Cod liver oil is a low dose vitamin D supplement with eicosapentaenoic acid and docosahexaenoic acid. A long tradition exists in Norway of taking cod liver oil during the winter to prevent vitamin D deficiency. Therefore, we initiated the Cod Liver Oil for Covid-19 Prevention Study (CLOC), where participants were randomised to receive cod liver oil or placebo (corn oil) during the winter of 2020-21, and we examined whether cod liver oil could prevent SARS-CoV-2 infection, serious covid-19, or other acute respiratory infections.

Methods

CLOC was a randomised, parallel group treatment, quadruple masked (participant, investigator, outcomes assessor, and data analysts), two armed trial. Supplement 1 provides details of the study protocol, changes to the protocol, and the statistical analysis plan. Ethical approval of the trial was obtained (30 September 2020), and the trial was registered in ClinicalTrials.gov (22 October 2020) before recruitment of participants. The statistical analysis plan was registered in ClinicalTrials.gov (26 November 2021) before the analysis and unblinding of the intervention.

Trial population

Between 10 November 2020 and 13 April 2021, adults (aged ≥18 years) with a Norwegian personal identity number and electronic access to the secure national digital governmental identification service, were invited to participate in the trial through a media campaign, and invitations were also sent to participants of the Norwegian Covid-19 Cohort Study. Excluded were people with prespecified diseases (including a history of renal failure or dialysis, hypercalcaemia, severe liver disease (cirrhosis), sarcoidosis, or other granulomatous diseases (eg, granulomatosis with polyangiitis (formerly Wegener's granulomatosis)), and previous covid-19), those who could not swallow oil, those with reactions to fish or cod liver oil, or corn oil, and those with indications for taking vitamin D supplements (vegan, pregnant, aged >75 years). Participants already taking cod liver oil or any other supplements with vitamin D for ≥5 days/week were also excluded, except for those with dark skin when all participants were included, regardless of their use of vitamin D supplements. Figure 1 shows the numbers of eligible randomised participants and the number of participants in the final analyses.

Intervention

Participants were randomised in a 1:1 ratio to take cod liver oil or placebo (corn oil). Both products were oils in liquid form, and the recommended daily dosage was 5 mL. The cod liver oil was flavoured with lemon, and the same lemon aroma was added to the placebo oil. Both products were successfully blind tested by an experienced taste panel (who could not distinguish between the products) who routinely blind test each batch of cod liver oil that is produced. Participants were encouraged to take the supplement at the same time each day, with a 5 mL measurement spoon or tablespoon, according to the instructions they received with the product (a picture of a tablespoon with 5 mL of oil). For the cod liver oil, 5 mL of oil contained about 10 µg of vitamin D_3 (400 IU), 1.2 g of long chained omega 3 polyunsaturated fatty acids, including 0.4 g of eicosapentaenoic acid and 0.5 g of docosahexaenoic acid, 250 µg of vitamin A, and 3 mg of vitamin E. For placebo, 5 mL of corn oil contained about 15.8 µg of vitamin A and 3.8 mg of vitamin E (analysed levels of vitamins A, D, and E in cod liver oil and placebo are presented in sTable 1, supplement 2).

Randomisation, blinding, and data storage

Randomisation was conducted at the Department of Research Support, Oslo University Hospital, by staff not involved in the trial (rounds of randomisation are listed in sTable 2, supplement 2). Randomisation was conducted as simple randomisation without blocking or stratification because of the large study population. A list of participants with their addresses and group assignment (cod liver oil or placebo group) was provided to a packaging company, which sent cod liver oil or placebo to participants. Staff at the packaging company were not involved in the trial, and no individuals involved in the trial had access to the list.

Participants and researchers involved in all phases of the trial were blinded to the group assignment of each participant. Unblinding was done when the analysis of all co-primary endpoints was completed (13 December 2021). The University of Oslo's services for sensitive data was used to collect, store, and analyse the data.

Questionnaires

Participants completed baseline questionnaires before they were randomised to receive cod liver oil or placebo. The questionnaires covered personal data, questions related to vitamin D, and other questions. After randomisation, practical instructions were sent to participants, followed by six (monthly) reporting questionnaires with items on compliance with the intervention, infection with SARS-CoV-2, acute respiratory infections, vaccination for covid-19, open question on side effects, and other questions (sAppendix 1, supplement 2). Compliance was defined as strict if >0.5 L of cod liver oil or placebo was consumed, or cod liver oil or placebo was taken for >2-3 months. Loose compliance was defined as >0.1 L of cod liver oil or placebo consumed, cod liver oil or placebo taken for >0-1 month, or cod liver oil or placebo consumed for >1 day/week. Total participation time for each participant was estimated from the earliest reported start date of taking cod liver oil or placebo
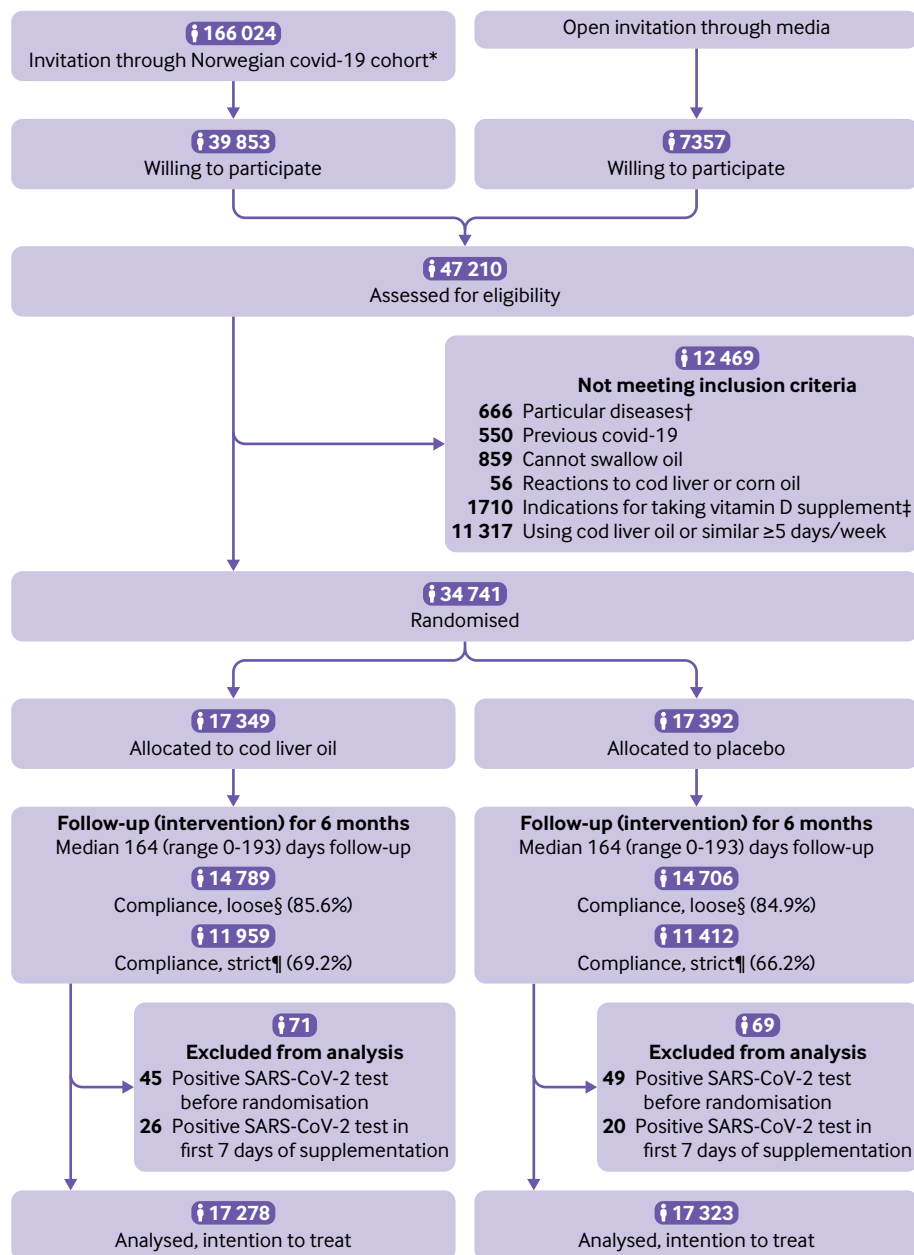
Fig 1 | Consolidated Standards of Reporting Trials (CONSORT) flow diagram of Cod Liver Oil for Covid-19 Prevention Study (CLOC). *Norwegian Covid-19 Cohort Study is a population based open cohort established in March 2020. †Including history of renal failure or dialysis, hypercalcaemia, severe liver disease (cirrhosis), sarcoidosis, or other granulomatous disease (eg, granulomatosis with polyangiitis (formerly Wegener's granulomatosis)). ‡Vegan, pregnant, ≥75 years old. §Loose compliance: reported consuming ›0.1 L of cod liver oil or placebo, consuming cod liver oil or placebo for ›0-1 month, or consuming cod liver oil or placebo for ›1 day/week. ¶Strict compliance: reported consuming ›0.5 L of cod liver oil or placebo or consuming cod liver oil or placebo for ›2-3 months

until the latest reported final date of taking cod liver oil or placebo. If no start date was reported, the start date was set as the randomisation date plus 14 days. If no final date was reported, the final date was set as the final date of the intervention period (2 June 2021). Side effects were categorised and graded according to the Common Terminology Criteria for Adverse Events (CTCAE). The first questionnaire was sent out on 21 December 2020 and the last on 2 June 2021. The University of Oslo's web based solution Nettskjema was used to distribute information and questionnaires electronically.

Endpoints: covid-19 and other acute respiratory infections

Four co-primary endpoints were assessed. The first co-primary endpoint was the incidence of a positive SARS-CoV-2 nasopharyngeal or oropharyngeal swab test determined by reverse transcriptase-quantitative polymerase chain reaction in a Norwegian microbiology laboratory reported to the Mandatory Norwegian Surveillance System for Communicable Diseases (MSIS). The second co-primary endpoint was the incidence of serious covid-19 (MSIS confirmed covid-19) with self-reported dyspnoea, or admission to

hospital or death. If data were missing for the variable serious covid-19, participants were included in the non-serious covid-19 outcome. Missing information was checked against medical records connected to the Norwegian Cause of Death Registry for information on deaths. The third co-primary endpoint was the incidence of participants with ≥1 negative SARS-CoV-2 test results recorded in MSIS. Most testing in Norway during the trial was conducted after participants showed symptoms of covid-19, and in our data >85% of negative test results were accompanied by symptoms. Thus having ≥1 negative SARS-CoV-2 test results was used as an indication of having ≥1 acute respiratory infections. The fourth co-primary endpoint was the incidence of participants reporting ≥1 acute respiratory infections. Missing information or unreported acute respiratory infections were included in the non-acute respiratory infections outcome during the intervention period.

Prespecified secondary endpoints and exploratory endpoints

Prespecified secondary endpoints (number of participants admitted to hospital for covid-19 and number of participants in the intensive care unit for covid-19) were self-reported. Exploratory endpoints included self-reported side effects, blinding of the study supplement, and change in blood levels of $25(OH)D_3$ and omega 3 index over the study period (from a subsample of the population).

Dried blood spot samples

To determine the effect of the intervention on levels of $25(OH)D_3$ and omega 3 fatty acids, capillary blood was collected at home using dried blood spot samples in a random subpopulation of participants. Samples were obtained before and during supplementation with cod liver oil or placebo and analysed by Vitas Analytical Services (Oslo). In total, 945 participants were sent kits twice, and 342 participants returned both kits. Participants received the kits by post and took a fasting blood sample from the fingertip, placing the drops of blood directly onto Whatman 903 Protein Saver dried blood spot cards. Participants then placed the dried blood spot card in a light and airproof Ziploc bag with a desiccant and posted it to Vitas Analytical services. Samples were stored at −80°C until analysed after the intervention period (2–9 months after the samples were collected).

For analyses of $25(OH)D_3$, dried blood spot punches (four circles, diameter 3.2 mm, with dried whole blood on certified paper) of human whole blood were diluted with water. After whole blood extraction, analytes were extracted with 2-propanol containing an internal standard. After analytes were extracted, samples were centrifuged through a filter plate for removal of whole blood debris. The eluate was injected into a Ultivo Triple Quadrupole liquid chromatography-mass spectrometer (Agilent Technologies, Santa Clara, CA), separated by a Kinetex 2.6 µm C18 100 Å liquid chromatography column 100×4.6 mm (Phenomenex, Torrance, CA)

(Vitas Analytical Services). The accuracy of the 25(OH)D_3 analyses was maintained by participation in the Vitamin D External Quality Assessment Scheme (DEQAS, sAppendix 2 and sTable 3, supplement 2).

For analyses of omega 3 fatty acids, two punches of human whole blood were methylated with sodium methylate. After methylation, fatty acid methyl esters were extracted with hexane. After thorough mixing and centrifugation, 3 µL of the aliquot were injected into a gas chromatography-flame ionisation detector. Gas chromatography-flame ionisation detection was performed with an Agilent HP 7890A Gas Chromatograph System (Agilent Technologies, Palo Alto, CA). The fatty acid methyl esters were separated on a TR-FRAME gas chromatography column (30 m×0.25 mm×0.25 µm film column) from Thermo Scientific (part No 260M142P) (Vitas Analytical Services).

Baseline SARS-CoV-2 antibody analysis

Randomly selected participants (n=1333) provided whole blood samples to a local laboratory for analysis of SARS-CoV-2 antibodies at baseline. A flow cytometer based method was used to identify IgG antibodies against SARS-CoV-2 derived recombinant antigens in residual sera.[18] Samples with antibodies against both the receptor binding domain and the spike protein of SARS-CoV-2 were considered positive.

Statistical analyses

Power calculation was done with the fallback method, used to adjust for multiple testing.[19] Sample size calculations were based on our own unpublished results from the Norwegian Covid-19 Cohort Study. A significance level (α) of 0.05 was divided between the four co-primary endpoints (0.03, 0.018, 0.001, and 0.001, respectively). The α levels were different because the four endpoints were weighted differently. The first and second (covid-19 related) co-primary endpoints were assigned an α value of 0.03 and 0.018, respectively. Based on an expected incidence of SARS-CoV-2 infection of 1%, a 20% reduction in the incidence of SARS-CoV-2 infection in the intervention group, and power of 70%, 65 000 participants were required for the first co-primary endpoint. Based on an incidence of serious covid-19 of 0.25%, a 40% reduction in serious covid-19 in the intervention group, and power of 70%, 67 000 participants were required for the second co-primary endpoint. Based on the expected frequency of acute respiratory infections of >30% and a threshold of 10% reduction in acute respiratory infections, 23 000 participants would need to be included for the third and fourth co-primary endpoints with a power of >95%. An α value of 0.001 was assigned for the third and fourth co-primary endpoints. Multiple comparison adjusted confidence intervals are reported for the four co-primary endpoints (97%, 98.2%, 99.9%, and 99.9% confidence intervals, respectively).

All four co-primary endpoints were analysed for the relative risk of acquiring a condition using cod liver oil versus placebo with the Wald test. The time to first

occurrence of the co-primary endpoints was plotted with the Kaplan-Meier approach. Logistic regression and the Wald test were used in the subgroup analyses (grouped by sex, age, body mass index, skin type, exposure to sun from July to October 2020, use of vitamin D supplements, vaccinated during the study period, consumers of fatty fish, and strict compliance) to assess the effect on the co-primary endpoints. These ad hoc subgroup analyses were chosen as they could affect (or cause a known difference in) levels of 25(OH)D$_3$ or omega 3 fatty acids, or affect the incidence of SARS-CoV-2 infection or serious covid-19.

The Wilcoxon signed rank test, the Mann-Whitney test, and linear regression were used to compare 25(OH)D$_3$ and omega 3 index levels and changes between and within the cod liver oil and placebo groups ($\alpha<0.05$). Pearson's χ^2 test was used to examine side effects and which treatment participants thought they were assigned to in the cod liver oil and placebo groups ($\alpha<0.05$).

Participant and public involvement
The Norwegian Covid-19 Cohort Study had a user panel that was involved in the planning of the CLOC study, including setting the research agenda and planning of the questionnaires.

Results
Trial participants
Overall, 34 741 men and women were randomised to receive cod liver oil or placebo, and 140 participants were excluded from the analyses because of a positive SARS-CoV-2 test result during the period from consent to participation up to seven days after starting to take cod liver oil or placebo. Participants took the supplement (cod liver oil or placebo) for a median of 164 days. We included 17 278 and 17 323 participants in the cod liver oil and placebo groups, respectively, in the analyses (fig 1).

More than half of participants were women (64.5%), mean age was 44.9 years, and mean body mass index was 26.1 at baseline (table 1). Most participants (75.5%) did not use vitamin D supplements before enrolling in the trial, 61.5% consumed fatty fish, and 39.8% reported ≤30 hours of exposure to the sun from July to October 2020. Subsample analyses for SARS-CoV-2 antibodies at baseline showed that 28 of 1333 participants (2.1%) had a positive antibody test result (data not shown). During the intervention, 35.6% of participants (6233 and 6097 in the cod liver oil and placebo groups, respectively) reported receiving ≥1 doses of a SARS-CoV-2 vaccine (table 1).

Cod liver oil and covid-19
In total, 455 participants had a positive SARS-CoV-2 test result, with similar event rates in the cod liver oil and placebo groups (227 (1.31%) and 228 (1.32%) respectively; relative risk 1.00, 97.0% confidence interval 0.82 to 1.22, table 2). The Kaplan-Meier curve showed similar rates of positive SARS-CoV-2 test results in the cod liver oil and placebo groups (fig 2).

Serious covid-19 was reported by 222 participants (121 (0.70%) in the cod liver oil group and 101 (0.58%) in the placebo group), all had dyspnoea, 17 were admitted to hospital (eight in the cod liver oil group and nine in the placebo group), and no participants died. Of those admitted to hospital for covid-19, eight participants (four in each group) were in the intensive care unit. The relative risk of serious covid-19 was 1.20 (98.2% confidence interval 0.87 to 1.65, table 2) for the cod liver oil group compared with the placebo group. Analyses stratified by sex, age, body mass index, exposure to sun from July to October 2020, use of vitamin D supplements, vaccinated during the study period, consumers of fatty fish, and strict compliance did not modify the effect of cod liver oil on having a positive SARS-CoV-2 test result or serious covid-19 (sTable 4, supplement 2).

Cod liver oil and acute respiratory infections
Our results showed that 17 111 participants had ≥1 negative SARS-CoV-2 test results, with similar event rates in the cod liver oil and placebo groups (8546 (49.46%) and 8565 (49.44%), respectively; relative risk 1.00, 99.9% confidence interval 0.97 to 1.04, table 2). One or more acute respiratory infections were reported by 7798 participants (3964 (22.94%) and 3834 (22.13%) participants in the cod liver oil and placebo groups, respectively). The relative risk of having ≥1 acute respiratory infections was 1.04 (99.9% confidence interval 0.97 to 1.11, table 2) for the cod liver oil group compared with the placebo group. Analyses stratified by sex, age, body mass index, skin type, exposure to sun from July to October 2020, use of vitamin D supplements, vaccinated during the study period, consumers of fatty fish, and strict compliance did not appear to modify the effect of cod liver oil on having ≥1 negative SARS-CoV-2 test results or ≥1 acute respiratory infections (sTable 5 and sTable 6, supplement 2).

Blood levels of 25(OH)D$_3$ and omega 3 index
Of 945 participants who were sent a dried blood spot kit for measuring levels of 25(OH)D$_3$ and omega 3 index before and while taking the supplements, 342 returned two complete samples (172 in the cod liver oil group and 170 in the placebo group, table 3). From the first to the second measurement, participants in the cod liver oil group had only slightly increased concentrations of 25(OH)D$_3$ (median 4.4 nmol/L, 25th to 75th centiles −14.4-23.3, P=0.06). Cod liver oil was observed to prevent a reduction in 25(OH)D$_3$ in winter, however, as found in the placebo group (−12.5 nmol/L, −24.1-4.1, P<0.001). In the cod liver oil group, the mean concentration of 25(OH)D$_3$ was increased by 15.0 nmol/L (95% confidence interval 8.8 to 21.2, P<0.001) and the omega 3 index by 1.9% (1.6 to 2.2, P<0.001) compared with the placebo group. Our results showed that 295 (86.3%) participants had concentrations of 25(OH)D$_3$ ≥50 nmol/L before the intervention period (before supplementation). During the intervention period (during supplementation), 155 (90.1%) and

Table 1 | Characteristics of participants at baseline, according to randomisation to cod liver oil or placebo group. Data are number (%) of participants unless stated otherwise

Characteristics	Overall (n=34 601)	Cod liver oil group (n=17 278)	Placebo group (n=17 323)
Sex:			
Women	22 346 (64.6)	11 161 (64.6)	11 185 (64.6)
Men	12 254 (35.4)	6117 (35.4)	6137 (35.4)
Mean (SD) age (years)	44.9 (13.4)	45.0 (13.5)	44.9 (13.4)
Mean (SD) body mass index	26.1 (4.7)	26.1 (4.7)	26.1 (4.7)
Smoking:			
Never	17 770 (51.4)	8906 (51.5)	8864 (51.2)
Past smoker	12 116 (35.0)	6025 (34.9)	6091 (35.2)
Current smoker	2687 (7.8)	1340 (7.8)	1347 (7.8)
Chronic disease*:			
No chronic disease	25 403 (73.4)	12 658 (73.3)	12 745 (73.6)
≥1 chronic diseases	7669 (22.2)	3852 (22.3)	3817 (22.0)
Parental ethnic origin:			
Europe	32 831 (94.9)	16 441 (95.2)	16 390 (94.6)
Asia	720 (2.1)	358 (2.1)	362 (2.1)
Africa	217 (0.6)	108 (0.6)	109 (0.6)
Other	576 (1.7)	268 (1.6)	308 (1.8)
Skin type:			
Easily burnt	6453 (18.7)	3270 (18.9)	3183 (18.4)
Sometimes burnt to never burnt	25 809 (74.6)	12 895 (74.7)	12 914 (74.6)
Naturally tanned	695 (2.0)	334 (1.9)	361 (2.1)
Sun exposure from July to October 2020:			
≤30 hours	13 752 (39.8)	6924 (40.1)	6828 (39.4)
>30 hours	20 197 (58.4)	10 035 (58.1)	10 162 (58.6)
Vitamin D supplement use†:			
No	26 130 (75.5)	13 092 (75.8)	13 038 (75.3)
Yes	7705 (22.3)	3799 (22.0)	3906 (22.5)
Fatty fish consumer‡:			
No	12 714 (36.7)	6381 (36.9)	6333 (36.6)
Yes	21 285 (61.5)	10 601 (61.4)	10 684 (61.7)
Household count:			
1	5351 (15.5)	2703 (15.6)	2648 (15.3)
2	10 981 (31.7)	5477 (31.7)	5504 (31.8)
≥3	16 979 (49.0)	8462 (48.9)	8517 (49.1)
Children in household:			
0	19 120 (55.3)	9549 (55.3)	9571 (55.3)
1	4943 (14.3)	2507 (14.5)	2436 (14.1)
≥2	9190 (26.5)	4543 (26.3)	4647 (26.8)
Education:			
Primary or lower secondary school§	895 (2.6)	466 (2.7)	429 (2.5)
Secondary school or vocational programmes¶	6988 (20.2)	3469 (20.1)	3519 (20.3)
Higher education	22 529 (65.1)	11 302 (65.4)	11 227 (64.8)
Household income (NOK):			
≤1 million	18 290 (52.9)	9108 (52.6)	9182 (53.0)
>1 million	14 444 (41.7)	7274 (42.1)	7170 (41.4)
Occupational status:			
Working or student	28 634 (82.8)	14 325 (82.9)	14 309 (82.6)
Retired	2566 (7.4)	1294 (7.5)	1272 (7.3)
Unemployed, sick leave, or social security	3253 (9.4)	1595 (9.2)	1658 (9.6)
Other	1352 (3.9)	668 (3.9)	684 (3.9)
Vaccinated during study period**	12 330 (35.6)	6233 (36.1)	6097 (35.2)

SD=standard deviation; NOK=Norwegian kroner (1 Kr; £0.09; €0.10; $0.10).
Data were missing for 1.7-5.4% of participants except for the variable education where data were missing for 11.9% of participants.
*One or more of these chronic conditions: heart disease, hypertension, lung disease, asthma, diabetes, cancer, and other, or treated with immunosuppressants.
†Taking vitamin D supplements (including cod liver oil) ≥5 days/week was an exclusion criterion but individuals with a lower frequency of use were included.
‡Consuming fatty fish ≥1-2 days/week or ≥1-3 slices of bread with fatty fish, or both.
§≤10 years of school (seven years of primary school, three years of lower secondary school).
¶Upper secondary school or vocational programmes (usually about three years).
**Reported having ≥1 SARS-CoV-2 vaccines during the intervention period.

123 (72.4%) participants in the cod liver oil and placebo groups, respectively, had concentrations ≥50 nmol/L.

Side effects and blinding of trial participants

Overall, 10.1% and 11.3% of participants in the cod liver oil and placebo groups, respectively, reported ≥1 side effects while taking the supplements during the intervention period (table 3). The most common side effects were mild gastrointestinal symptoms, classified as CTCAE grade 1. The side effects classified as CTCAE grade 2 were more common in the placebo group than in the cod liver oil group because of a higher number of participants self-reporting low levels of vitamin D in the placebo group (n=36 v n=8, respectively). The other CTCAE grade 2 symptoms reported by participants were similarly distributed in the cod liver oil (n=13) and placebo (n=11) groups and included heart palpitations, allergic reactions, and gastrointestinal symptoms.

In the last reporting questionnaire, 17 860 (51.6%) participants responded to the question about which type of supplement they thought they had been taking. Of these, 7220 (78.6%) in the cod liver oil group and 7616 (87.8%) in the placebo group believed they had been taking a placebo or did not know, whereas 1966 (21.4%) in the cod liver oil group and 1058 (12.2%) in the placebo group thought they had been taking cod liver oil (table 3).

Discussion

In this large, randomised, primary prevention trial, supplementation with cod liver oil, a low dose vitamin D supplement, was not associated with a reduction in the incidence of SARS-CoV-2 infection, serious covid-19, or other acute respiratory infections compared with placebo. Only low grade side effects were reported, and fewer participants reported side effects in the cod liver oil group than in the placebo group.

The null findings of supplementation with cod liver oil on the risk of SARS-CoV-2 infection and serious covid-19 in our trial are in line with the results of a mendelian randomisation study that found no association between concentrations of 25(OH)D$_3$ and risk of SARS-CoV-2 infection or serious covid-19, and the authors concluded that vitamin D supplementation would have had no preventive effect.[7] Our findings are also similar to the results of a large trial on vitamin D and covid-19 (published as a preprint), although participants had lower levels of 25(OH)D$_3$ initially than our subsample and were given larger vitamin D doses.[20] Our null findings contrast with a recent small double blind, placebo controlled trial suggesting that vitamin D supplements prevented covid-19 in people at high risk of SARS-CoV-2 infection.[21] The supplementation regimen differed from ours, however, with 4000 IU of vitamin D given every day for one month, and 67% of participants had 25(OH)D$_3$ concentrations <50 nmol/L at the start of the trial. Our null findings also contrast our own unpublished, preliminary unadjusted results (Norwegian Covid-19 Cohort Study), indicating that participants taking cod liver oil had a reduced risk of developing covid-19 and of being admitted to hospital for covid-19 than non-users. Furthermore, two large observational studies (app based European/

Table 2 | Absolute and relative risk, and confidence intervals, for first, second, third, and fourth co-primary endpoints, according to randomisation to cod liver oil or placebo group, in intention-to-treat analyses

Co-primary endpoint	Overall (n=34 601) No (%)	Cod liver oil group (n=17 278)		Placebo group (n=17 323)		Relative risk (CI*)	P value†
		No	Absolute risk (% (CI*))	No	Absolute risk (% (CI*))		
Covid-19							
First: SARS-CoV-2 positive test result	455 (1.32)	227	1.31 (1.13 to 1.50)	228	1.32 (1.13 to 1.50)	1.00 (0.82 to 1.22)	0.98
Second: serious covid-19‡	222 (0.64)	121	0.70 (0.55 to 0.85)	101	0.58 (0.45 to 0.72)	1.20 (0.87 to 1.65)	0.17
Acute respiratory infections							
Third: ≥1 SARS-CoV-2 negative test results	17 111 (49.45)	8546	49.46 (48.21 to 50.71)	8565	49.44 (48.19 to 50.69)	1.00 (0.97 to 1.04)	0.97
Fourth: ≥1 self-reported acute respiratory infections	7798 (22.54)	3964	22.94 (21.89 to 24.00)	3834	22.13 (21.09 to 23.17)	1.04 (0.97 to 1.11)	0.07

*First and second co-primary endpoints (covid-19), 97.0% and 98.2% confidence interval, respectively; third and fourth co-primary endpoints (acute respiratory infections), 99.9% confidence interval.
†Logistic procedure P value for difference between cod liver oil and placebo groups determined with the Wald test.
‡SARS-CoV-2 positive test result and self-reported dyspnoea (n=222), admission to hospital (n=17, eight in the cod liver oil group and nine in the placebo group), or death (n=0). Data were missing for n=17 (13 in the cod liver oil group and four in the placebo group) for the variable serious covid-19; these were included in the non-serious covid-19 outcome.

American study and UK Biobank study) have found an association between the use of vitamin D supplements and reduced risk of covid-19.[1 22]

A meta-analysis described a protective effect of vitamin D supplements against acute respiratory infections, with the strongest effect for supplement doses of 400-1000 IU/day for up to a year.[12] In a more recent meta-analysis (published as a preprint), however, the authors questioned these initial results because clustering was not accounted for in one of the cluster randomised controlled trials included. The authors did a secondary analysis of these data, accounting for clustering in the randomised controlled trial, and the updated meta-analysis showed no protective effect of vitamin D supplements on acute respiratory infections.[23] This finding is in line with our trial of no protective effect of vitamin D supplementation against acute respiratory infections.

In our trial, 86% of participants in the subsample with dried blood spot tests had $25(OH)D_3$ concentrations ≥50 nmol/L before the trial started, which has been suggested as an adequate level.[23-25] Thus the possibility that an effect of supplementation with vitamin D on the risk of SARS-CoV-2 infection, serious covid-19, and other acute respiratory infections was missed because of adequate concentrations of $25(OH)D_3$ at the start of

the trial cannot be excluded. We found no difference in the relative risk for the co-primary endpoints when stratified by factors associated with levels of $25(OH)D_3$ before the trial started (such as exposure to sun from July to October 2020, use of vitamin D supplements, and consuming fatty fish). The proportion of participants with vitamin D deficiency ($25(OH)D_3$ <30 nmol/L) in the trial population, however, was most likely low. Our trial had a practical and realistic approach to supplementation with vitamin D for the prevention of covid-19 and other acute respiratory infections, testing whether those not taking vitamin D supplements at the start of the trial would benefit from supplementation during the winter. Widespread testing for $25(OH)D_3$ levels and only providing supplements for those with low levels would require a different study design.

Few studies have examined omega 3 fatty acids and the risk of SARS-CoV-2 infection, serious covid-19, and other acute respiratory infections. The NutriNet-Santé cohort found no association between dietary intake of omega 3 fatty acids and susceptibility to covid-19.[26] Conversely, others have seen an association between omega 3 supplementation and reduced risk of covid-19[1] and upper respiratory tract infections.[27] Also, an inverse association between the omega 3 index in blood and death from covid-19 has been reported.[28] The effect of vitamins A and E, also present in cod liver oil, on SARS-CoV-2 infection, serious covid-19, and other acute respiratory infections is not known. Vitamin D has been suggested to reduce the risk of severe asthma exacerbations in those with mild to moderate asthma,[29] whereas a Norwegian study found an increase in the incidence of adult onset asthma with the use of cod liver oil.[30] Vitamin A levels in the cod liver oil used in that study were more than three times the daily amount provided by the cod liver oil in our study, however. We believe it is unlikely that vitamin A or E would have masked any effect of vitamin D or omega-3 fatty acids on covid-19 disease or other acute respiratory infections.[1 31]

Only low grade side effects were reported in our trial. More side effects were reported by participants in the placebo group than in the cod liver oil group, including low levels of vitamin D.
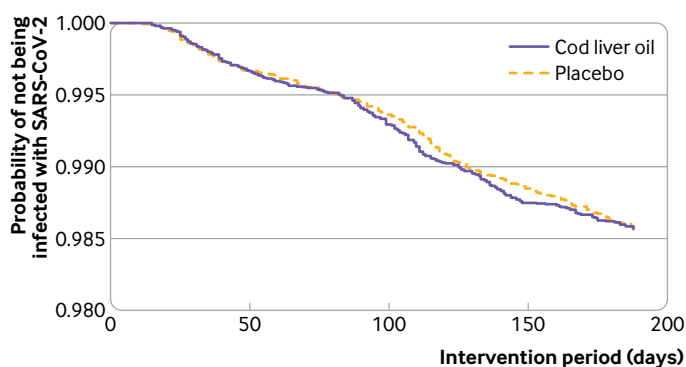

Fig 2 | Kaplan-Meier plot of the probability of a positive SARS-CoV-2 test result for participants in the cod liver oil (n=17 278) and placebo (n=17 323) groups during the intervention period

Table 3 | Exploratory endpoints; side effects, blinding, and measured compliance according to randomisation to cod liver oil or placebo group. Data are median (25th to 75th centiles) or number (%) unless stated otherwise

	Overall (n=34 601)	Cod liver oil group (n=17 278)	Placebo group (n=17 323)	P value
Measured compliance, from dried blood spots of a subsample*				
25-hydroxyvitamin D$_3$ (nmol/L):				
Before supplementation	70.5 (56.7-92.3)	66.9 (52.2-91.0)	73.3 (59.6-92.7)	0.04†
During supplementation	67.9 (54.0-85.6)	74.1 (60.1-88.0)	62.8 (48.2-81.6)	<0.001†
Change	−3.6 (−20.9-14.4)	4.4 (−14.4-23.3)	−12.5 (−24.1-4.1)‡‡	<0.001†
Omega 3 index (%):				
Before supplementation	4.6 (3.7-5.7)	4.6 (3.7-5.5)	4.6 (3.7-5.8)	0.62†
During supplementation	5.1 (3.8-6.5)	6.2 (5.3-7.4)	4.1 (3.4-5.0)	<0.001†
Change	0.3 (−0.7-1.7)	1.5 (0.4-2.5)‡‡	−0.5 (−1.1-0.1)‡‡	<0.001†
Side effects				
Reported ≥1 side effects	3708 (10.7)	1742 (10.1)	1966 (11.3)	<0.001‡
Grade§:				
1	3640 (10.5)	1721 (10.0)	1919 (11.1)	<0.001‡
2¶	68 (0.2)	21 (0.1)	47 (0.3)	0.002‡
Most commonly self-reported:				
Nausea, vomiting	1519 (4.4)	694 (4.0)	825 (4.8)	<0.001‡
Regurgitation, burping	854 (2.5)	420 (2.4)	434 (2.5)	0.68‡
Stomach symptoms**	826 (2.4)	369 (2.1)	457 (2.6)	0.002‡
Reflux	772 (2.2)	384 (2.2)	388 (2.2)	0.94‡
Response to question "What supplement did you think you were taking?"††				
Cod liver oil	3024 (16.9)	1966 (21.4)	1058 (12.2)	<0.001‡
Placebo	11 458 (64.2)	5364 (58.4)	6094 (70.3)	<0.001‡
Do not know	3378 (18.9)	1856 (20.2)	1522 (17.5)	<0.001‡

*Subsample of 342 participants (cod liver oil group n=172, placebo group n=170) with dried blood spot samples from before randomisation (before supplementation) and during the intervention period (during supplementation).
†Mann-Whitney U test, cod liver oil versus placebo group.
‡Pearson x^2 test, cod liver oil versus placebo group.
§Graded according to the Common Terminology Criteria for Adverse Events (CTCAE).
¶Of the CTCAE grade 2 side effects, self-reported low vitamin D levels were n=8 in the cod liver group and n=36 in the placebo group.
**Stomach symptoms included stomach pain, diarrhoea, and constipation.
††Answered by 17 860 (51.6%) participants.
‡‡Significant change (Wilcoxon signed rank test) from before to during supplementation, P<0.001.

Strengths and limitations of this trial

Limitations of our trial include self-reported data for two of the four endpoints: dyspnoea, defining serious covid-19, and self-reported acute respiratory infections. Self-experienced symptoms are important for participants, however, and the objective MSIS based endpoints corroborated these results. Compliance was self-reported, and therefore we had no data on compliance from participants not returning our questionnaires. Hence we could not distinguish between not responding to the questionnaires and being compliant with the intervention. However, the results were comparable for the strict compliant subgroup and the whole trial population.

The median intervention time was relatively short at 164 days, and we do not have data on the possible longer term effects of cod liver oil. Also, we could not distinguish between the potential effect of vitamin D and eicosapentaenoic acid or docosahexaenoic acid, or explore a dose-response relation. Concentrations of 25(OH)D$_3$ and omega-3 index were only available for a small subsample of participants and thus we could not study how levels of 25(OH)D$_3$ at the start of the trial were related to the risk of SARS-CoV-2 infection and other acute respiratory infections. The available 25(OH)D$_3$ results indicated that our trial population seemed to have adequate levels of 25(OH)D$_3$ at inclusion in the trial.

We aimed to include 80 000 participants in our trial but we could only include 34 601. Because of fewer covid-19 incident cases than expected (455 instead of 650), the trial was slightly underpowered to detect a 20% reduction in the incidence of SARS-CoV-2 infection. Serious covid-19 was more prevalent than anticipated, however, and the trial was adequately powered for the second, third and fourth co-primary endpoint despite the reduced numbers of participants enrolled in the trial.

Our trial had several strengths, including a large general adult population that did not use vitamin D supplements regularly before the start of the trial, implying that our vitamin D supplementation regimen was realistic for testing whether this population would benefit from supplements during the winter. We found good compliance with the study supplement among participants, and blinding was successful because most participants thought they had been taking a placebo or did not know. The SARS-CoV-2 swab test, analysed by reverse transcriptase-quantitative polymerase chain reaction in an accredited Norwegian microbiology laboratory, was the basis for the first and third co-primary endpoints (positive and negative tests, respectively). Also, analysis of SARS-CoV-2 antibodies in a subsample of participants at baseline confirmed that only 2.1% had been infected with SARS-CoV-2 previously.

Conclusions

Daily supplementation with cod liver oil, a low dose vitamin D, eicosapentaenoic acid, and docosahexaenoic

acid supplement, for six months during the SARS-CoV-2 pandemic among Norwegian adults, did not reduce the incidence of SARS-CoV-2 infection, serious covid-19, or other acute respiratory infections. Only low grade side effects were reported.

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Web appendix 1: Supplement 1—study protocol and data analysis plan

Web appendix 2: Supplement 2—supplemental online content