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The Effects of Vitamin D and Marine Omega-3 Fatty Acid Supplementation on Chronic Knee Pain in Older US Adults: Results From a Randomized Trial

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Objective. Knee pain from osteoarthritis is frequent in the adult population. Prior trials have had conflicting results concerning the therapeutic effects of vitamin D on knee pain, and few trials have investigated marine omega-3 fatty acids (n-3 FA).

Methods. In the double-blind, placebo-controlled Vitamin D and Omega-3 Trial (VITAL), 25,871 US adults were randomized in a 2-by-2 factorial design to receive vitamin D or n-3 FA. We identified a subgroup with chronic knee pain prior to randomization and assessed knee pain at baseline and annually during follow-up using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (graded on a 0–100 scale, where 100 indicates worst symptoms). Repeated measures modeling was used to test the effect of randomized treatment on WOMAC pain scores over follow-up after adjustment for age and sex. Analyses were repeated for WOMAC function and stiffness.

Results. This study included 1,398 participants who returned at least one knee pain questionnaire. The mean age was 67.7 years, 66% were women, and the mean ± SD WOMAC pain score was 37 ± 19. The mean ± SD follow-up time was 5.3 ± 0.7 years. WOMAC pain did not differ between the active vitamin D group and the vitamin D placebo group or between the active n-3 FA group and the n-3 FA placebo group at any time point during follow-up. Linear time-by-treatment interactions were not significant for either treatment (vitamin D, $P = 0.41$; n-3 FA, $P = 0.77$). Vitamin D and n-3 FA supplementation did not significantly affect WOMAC function or stiffness scores over time.

Conclusion. Our findings indicate that vitamin D and n-3 FA supplementation for a mean of 5.3 years does not reduce knee pain or improve function or stiffness in a large sample of US adults with chronic knee pain.

INTRODUCTION

Osteoarthritis (OA) is a frequent cause of knee pain in older adults, with symptomatic knee OA affecting ~14 million in the US (1,2). An estimated 25% of older adults have knee pain, making knee symptoms one of the most common reasons patients seek outpatient care (3,4). Despite the increasing prevalence of symptomatic knee OA, medical therapies are largely limited to exercise, weight management, and medications for pain control that may confer adverse effects (5,6). Identifying safe and inexpensive therapies that reduce pain could vastly improve management of chronic knee pain. Recent interest has focused on the role of vitamin D in OA due to its role in bone resorption and muscle strength, as well as its antiinflammatory properties (7–9). Marine omega-3 fatty acids (n-3 FA), found in fish oils, have also been touted for their ability to mitigate inflammation and the catabolic environment that promotes cartilage degradation (10,11).

While prospective studies have demonstrated an association of lower vitamin D levels with pain and OA progression, several randomized controlled trials (RCTs) of vitamin D supplementation

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for the treatment of OA have yielded conflicting results (12–17). Sanghi et al conducted a 1-year trial of vitamin D versus placebo in patients with symptomatic knee OA and vitamin D insufficiency and reported a small, but significant, improvement in pain and function in patients receiving vitamin D (16). Conversely, a 2-year RCT of vitamin D supplements in participants with symptomatic knee OA did not demonstrate significant differences in pain or cartilage volume (15), nor did a subsequent 3-year RCT demonstrate any benefits of vitamin D for joint space narrowing or pain (17). Fewer RCTs have been conducted using fish oil, although Hill et al conducted a 2-year RCT of low-dose versus high-dose fish oil in patients with symptomatic knee OA and demonstrated that participants receiving low-dose fish oil had significantly lower pain scores at 18 and 24 months compared to participants in the high-dose group; however, no placebo group was analyzed (18).

The Vitamin D and Omega-3 Trial (VITAL), a large, nationwide, population-based trial with 5 years of randomized intervention, provides a unique opportunity to investigate the potential role of vitamin D and fish oil on knee pain (19,20). We aimed to test the long-term effects of vitamin D and n-3 FA supplements on chronic knee pain, hypothesizing that we would discover prolonged beneficial effects of both vitamin D and n-3 FA supplements on the severity of knee pain.

PATIENTS AND METHODS

Study population. The VITAL trial is a completed randomized, double-blind, placebo-controlled clinical trial performed to assess the effects of vitamin D and n-3 FA supplements in the primary prevention of cancer and cardiovascular disease (21). The VITAL included 25,871 US adults without cancer or cardiovascular disease at the time of enrollment. Men age ≥50 years and women age ≥55 years were recruited from the community beginning in March 2011 through brochures, targeted mailings, media reports, and advertisements. African American participants were oversampled (20%; n = 5,106) (21). Participants were randomized to receive vitamin D_3 (cholecalciferol; 2,000 IU/day), marine n-3 FA (Omacor 1 gm/day, consisting of 840 mg eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA] in a 1.3:1 ratio), or placebo in a 2-by-2 factorial design (21). Participants were required to limit the amount of personal supplements of vitamin D taken to 800 IU/day and calcium to 1,200 mg/day, and to avoid fish oil supplements. Randomization was computer generated in blocks of 8 stratified by sex, race, and 5-year age groups (21).

Participants in the parent trial were mailed follow-up questionnaires at 6 months and then annually. The mean follow-up time was 5.3 years (range 3.8–6.1 years). The details of this trial have been published previously (19–22). Within a subcohort of the VITAL trial for whom blood samples were available, the mean ± SD 25-hydroxyvitamin D level at baseline was 30.8 ± 10 ng/ml, and the mean ± SD n-3 FA index was 2.7 ± 0.9% (19,20). In participants randomized to receive vitamin D who provided baseline and 1-year blood samples, vitamin D levels increased by 12 ng/ml (19). Adherence was defined based on participant reports of taking at least two-thirds of the study medication. For vitamin D, the adherence rate was 82.0% for the treatment group and 80.3% for the placebo group (19). For n-3 FA, the adherence rate was 81.6% for the treatment group and 81.5% for the placebo group (20).

We identified a knee pain cohort consisting of VITAL participants who self-reported frequent and chronic knee pain at enrollment prior to randomization and who were highly likely to have knee OA based on an affirmative response to all of the following: 1) self-reported knee pain symptoms in walking 2–3 blocks; 2) knee pain >1 day/week; 3) knee pain for ≥1 year; and 4) a physician's diagnosis of knee OA. These questions were chosen based on the development of a knee OA screening instrument with enhanced specificity by LaValley et al, which included pain in the past month in walking 2–3 blocks and reports of ever having a physician diagnosis of knee OA (23). This instrument proved particularly specific for knee OA, with a specificity of 94% and a sensitivity of 46% (23). We additionally queried about the duration and frequency of knee symptoms, as we considered it important to investigate a group with active and persistent knee symptoms. We performed a medical record review on a subset of knee pain participants to validate OA as outlined below. Patients with previous bilateral total knee replacement (TKR) were excluded. For participants with prior unilateral TKR, the native contralateral knee was eligible for inclusion. Assuming an SD of 18 points for the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score, we had 93% power to detect an effect size of 0.2.

During the VITAL trial run-in period (baseline), 1,430 trial participants (6%) were eligible for the knee pain cohort and were mailed supplementary baseline knee pain questionnaires. Of the participants eligible for the knee pain cohort, 1,221 (85%) returned a baseline questionnaire. In year 2 of the trial, an additional 186 participants were identified as eligible for the knee pain cohort due to delayed baseline questionnaire return and were sent questionnaires; 177 of these participants returned the questionnaire, providing additional post-randomization data. There were no differences in age, race, or geographic location between the final 1,398 participants in the knee pain cohort and the 218 participants eligible at enrollment who did not return any knee pain questionnaires. Participants who did not return questionnaires had a higher body mass index (BMI) (34 kg/m^2 versus 32 kg/m^2) and were more likely to be female (74% versus 66%). Eighty-six participants completed the baseline knee pain questionnaire, but none of the follow-up questionnaires.

The VITAL trial is registered with ClinicalTrials.gov (ClinicalTrials.gov identifier: NCT01169259 for the parent trial and ClinicalTrials.gov identifier: NCT01351805 for the knee pain ancillary study). This study was approved by the Partners' Human Research Committee.

Data elements at baseline. Participants' demographic and socioeconomic characteristics, including age, sex, race/ethnicity, geographic location of residence, level of education, and

income, were collected on the baseline survey. BMI (in kg/m^2, calculated from self-reported weight and height), and self-reported physical activity (metabolic equivalent [MET] hours/week) were also recorded.

Knee symptom assessment. Participants in the knee pain cohort completed a knee symptom questionnaire at baseline and annually thereafter on VITAL follow-up questionnaires based on their most painful knee during the last week, or both knees, if both were equally painful. Participants were randomized from November 2011 through March 2014. The trial intervention ended on December 31, 2017. The mean ± SD follow-up time for the knee pain cohort was 5.3 ± 0.7 years, and on average, each participant completed 3.8 ± 1.3 questionnaires out of a possible 6. We assessed patient-reported knee symptoms using the WOMAC pain and stiffness subscales and a modified version of the WOMAC function subscale (24,25).

The modified WOMAC function scale reduces redundancy by compressing the function scale from 17 items to 7 items (ascending stairs, rising from sitting, walking on a flat surface, getting in/out of car, putting on socks/stockings, rising from bed, and sitting), yet has a Cronbach's alpha of 0.87–0.93 and retains responsiveness (25). The WOMAC pain and stiffness scales were used in their original format. WOMAC subscale scores were individually summed and scaled (0–100, where 100 indicates worst). A WOMAC end-user license was obtained for this study. Knee pain frequency was assessed as follows: never, <1 day/week, 1–2 days/week, 3–6 days/week, and daily. Frequency of use of the following medications was collected at baseline and during follow-up: 1) acetaminophen; 2) nonsteroidal antiinflammatory drugs, including naproxen, cyclooxygenase 2 inhibitors, indomethacin, etodolac, ibuprofen, nabumetone, diclofenac, salsalate, and piroxicam; and 3) analgesics, including opioids (morphine, propoxyphene, oxycodone, hydrocodone, tramadol, amitriptyline, butalbital, and gabapentin). Prevalent TKR was assessed at baseline and incident TKR was ascertained during follow-up.

Validation. Validation of OA as the primary cause of knee pain as well as a subset of patient-reported TKR has been previously reported (26). Briefly, of the 1,398 participants in the knee pain cohort, medical records were requested from 200 men and 200 women (29% randomly selected) in order to validate OA. We were able to obtain medical records for 226 of these 400 participants (57%) for review. Of the participants for whom medical records were received, 207 (92% of those reviewed) were confirmed to have knee OA based on physician's diagnosis and/or radiographic imaging. Of the 174 participants for whom we were unable to obtain medical records, the majority were due to the inability to contact either the participant or the treating physician (103 records). Additionally, 32 participants did not provide written consent, and 15 could not provide records for miscellaneous reasons, including destruction of older records. Only 24 participants denied being treated or evaluated for knee OA (26).

Statistical analysis. Baseline characteristics were assessed using means and percentages. We used an intent-to-treat analysis and included eligible participants who returned ≥1 of the knee pain questionnaires over the trial period. We first examined the relationship between change in the WOMAC pain score over the study duration (dependent variable) and treatment arm (independent variable). For the primary analysis, we used a repeated measures model with unstructured variance to assess the effect of treatment arm on measures of WOMAC pain at follow-up after adjustment for age, sex, and the other treatment arm. The linear time-by-treatment interaction term was used to assess change in WOMAC pain over time among participants randomized to receive treatment versus placebo. While the primary analysis examined the trend in the treatment effect over time, we also considered the treatment difference at each time point. In this factorial trial, we assessed the main effects of the treatment arms, comparing all participants randomized to receive vitamin D to those randomized to receive vitamin D placebo, regardless of n-3 FA status; and similarly, comparing all participants randomized to receive n-3 FA to those randomized to receive n-3 FA placebo, regardless of vitamin D status. Because undergoing TKR during the trial period likely would have influenced self-reported knee symptoms, participants were censored at TKR. The WOMAC pain score was the prespecified primary outcome measure, but the above analyses were also repeated for the WOMAC function and WOMAC stiffness subscales.

We evaluated the effect of vitamin D and n-3 FA supplements on WOMAC pain over time in several subgroups after stratifying by sex, race (white versus non-white), BMI (<27 versus ≥27), baseline fish consumption (<1.5 servings/week versus ≥1.5 servings/week), and other treatment (i.e., randomized to receive n-3 FA and stratified by active vitamin D treatment versus placebo vitamin D). In the subset of participants with measured baseline vitamin D levels (n = 854) and n-3 FA index (n = 840), we assessed effect modification based on vitamin D level <20 ng/ml and ≥20 ng/ml and n-3 FA index <2.7% and ≥2.7%. We again used a repeated measures model with censoring for TKR. We adjusted for age, sex, and other treatment arm in analyses in which covariates were not regarded as potential modifiers.

We conducted 2 sensitivity analyses. First, we included only the participants who completed baseline prerandomization knee pain questionnaires (n = 1,221). Censoring at TKR may introduce bias, as those who undergo TKR typically do so in response to increased pain. Thus, in a second sensitivity analysis, we assumed a worst-case scenario and added 10 points to the last-reported WOMAC pain score prior to receipt of TKR and imputed this value as the next score after TKR, with subsequent scores kept as missing. Prior work by Collins et al demonstrates that pain trajectories are relatively stable over a 6-year period (i.e., patients

with high pain continue to have high pain), which supports this assumption (27).

We examined 2 secondary outcome measures. The risk of incident TKR in each treatment arm was evaluated using Cox proportional hazards regression after adjustment for age and sex. We also assessed change in the use of analgesics, including opioids, as defined above, over the study duration. Use of analgesics, including opioids, was dichotomized as occasional or daily versus no use. General estimating equations, with adjustment for age, sex, and censoring for TKR, were created to examine whether use of vitamin D or n-3 FA modified the use of analgesics, including opioids, over time.

A 2-tailed *P* value less than 0.05 was considered significant; we did not adjust for multiple comparisons. All analyses were performed using SAS 9.4 statistical software.

RESULTS

Baseline characteristics. In the knee pain cohort, 674 participants (48%) were randomized to receive active vitamin D and 695 participants (50%) to receive active n-3 FA. The baseline demographic characteristics of the knee pain cohort, overall and stratified by treatment group, are shown in Table 1. Further knee pain–related baseline characteristics collected on the knee symptom questionnaire are shown by treatment group in Table 2. A small percentage of patients (5.4%) who reported having at least daily knee pain on the screening questionnaire subsequently reported no or less than daily symptoms once enrolled in the knee pain cohort but were included in the analysis. Baseline characteristics were well balanced between those randomized to receive active vitamin D and those randomized to receive vitamin D placebo. For n-3 FA, those randomized to receive placebo reported higher physical activity levels (11 MET hours/week versus 7 MET hours/week) and differences in the frequency of acetaminophen usage. There were no differences in baseline WOMAC subscales between the active vitamin D group and the vitamin D placebo group or between the active n-3 FA group and the n-3 FA placebo group.

Primary outcome measure. Over the course of the trial, 61 participants (4%) in the knee pain cohort died. Of these, 26 were receiving vitamin D, 35 were receiving vitamin D placebo, 37 were receiving n-3 FA, and 24 were receiving n-3 FA placebo. During the follow-up period for the knee pain cohort, 296 participants (21%) reported having a TKR. Of these participants, 140 were receiving active vitamin D and 156 were receiving vitamin D placebo; 146 were receiving active n-3 FA and 150 were receiving n-3 FA placebo. No deaths were attributable to the interventions.

Vitamin D. After adjustment for age and sex, the least squares mean ± SEM WOMAC pain score at baseline was 35.4 ± 0.7 in the active vitamin D group and 36.5 ± 0.7 in the vitamin D placebo group. At the time of last follow-up,

Table 1. Baseline characteristics of the knee pain cohort within the VITAL*

		Vitamin D		Omega-3 fatty acid	
	Knee pain cohort (n = 1,398)	Active (n = 674)	Placebo (n = 724)	Active (n = 695)	Placebo (n = 703)
Age, mean ± SD, years	67.7 ± 6.9	67.5 ± 6.7	68.0 ± 7.13	67.9 ± 7.0	67.6 ± 6.8
BMI, mean ± SD kg/m^2	31.8 ± 7.5	31.8 ± 7.0	31.8 ± 7.9	32.0 ± 7.1	31.5 ± 7.8
Physical activity, total MET hours/week, median (IQR)	8.5 (1.9–23.2)	7.6 (2.0–21.8)	10.0 (1.9–24.5)	7.2 (1.7–21.5)	10.6 (2.1–25.0)
Female	920 (66)	439 (65)	481 (66)	464 (67)	456 (65)
Race					
Non-Hispanic white	882 (65)	422 (64)	460 (65)	436 (64)	446 (65)
African American	375 (28)	182 (28)	193 (27)	197 (29)	178 (26)
Hispanic	50 (4)	24 (4)	26 (4)	16 (2)	34 (5)
Other/unknown	59 (4)	29 (4)	30 (4)	29 (4)	30 (4)
Geographic location					
Northeast	335 (24)	155 (23)	180 (25)	152 (22)	183 (26)
Midwest	337 (24)	157 (23)	180 (25)	171 (25)	166 (24)
West	325 (23)	163 (24)	162 (22)	168 (24)	157 (22)
Southeast	401 (29)	199 (30)	202 (28)	204 (29)	197 (28)
Education level					
≤High school diploma or GED	274 (20)	127 (19)	147 (20)	140 (20)	134 (19)
>High school	1,118 (80)	543 (81)	575 (80)	551 (80)	567 (81)
Annual income level					
<$50,000	619 (49)	298 (49)	321 (49)	321 (51)	298 (47)
≥$50,000	646 (51)	316 (52)	330 (51)	308 (49)	338 (53)

* Except where indicated otherwise, values are the number (%). VITAL = Vitamin D and Omega-3 Trial; BMI = body mass index; MET = metabolic equivalent task; IQR = interquartile range; GED = general education development.

Table 2. Baseline knee pain characteristics in the knee pain cohort*

		Vitamin D		Omega-3 fatty acid	
	Knee pain cohort (n = 1,221)	Active (n = 591)	Placebo (n = 630)	Active (n = 595)	Placebo (n = 626)
Knee pain frequency, no. (%)					
Never	5 (0.4)	2 (0.3)	3 (0.5)	2 (0.3)	3 (0.5)
<1 day/week	62 (5)	32 (6)	40 (7)	35 (6)	27 (4)
1–2 days/week	136 (12)	75 (13)	61 (10)	71 (12)	65 (11)
3–6 days/week	240 (20)	115 (20)	125 (21)	115 (20)	125 (21)
Daily	736 (62)	350 (61)	386 (64)	354 (61)	382 (63)
Prior total knee replacement, no. (%)	176 (15)	89 (15)	87 (14)	83 (14)	93 (15)
Unilateral knee pain, no. (%)	821 (71)	400 (71)	421 (71)	398 (70)	423 (71)
Bilateral knee pain, no. (%)	338 (29)	164 (29)	174 (29)	168 (30)	170 (29)
Acetaminophen use, no. (%)					
Never	525 (49)	243 (47)	282 (51)	245 (48)	280 (51)
Occasionally	380 (36)	186 (36)	194 (35)	175 (34)	205 (37)
Daily	162 (15)	83 (16)	79 (14)	94 (18)	68 (12)
Nonsteroidal antiinflammatory medication use, no. (%)					
Never	269 (24)	135 (25)	134 (23)	127 (23)	142 (25)
Occasionally	391 (35)	192 (35)	199 (35)	199 (37)	192 (33)
Daily	462 (41)	221 (40)	241 (42)	217 (40)	245 (42)
Analgesics, including opioids, no. (%)					
Never	762 (69)	364 (68)	398 (70)	371 (69)	391 (68)
Occasionally	171 (15)	87 (16)	84 (15)	81 (15)	90 (16)
Daily	172 (16)	86 (16)	86 (15)	82 (15)	90 (16)
WOMAC pain	36.7 ± 18.7	36.2 ± 18.5	37.0 ± 18.8	37.2 ± 18.5	36.1 ± 18.8
WOMAC function	36.7 ± 19.9	36.0 ± 19.8	37.5 ± 20.0	37.0 ± 20.2	36.4 ± 19.6
WOMAC stiffness	44.3 ± 21.8	43.5 ± 22.1	45.0 ± 21.6	45.0 ± 22.5	43.7 ± 21.3

* Except where indicated otherwise, values are the mean ± SD. Data are from a baseline knee pain questionnaire after eligibility was established; thus, some included participants now report having no or infrequent knee pain. WOMAC = Western Ontario and McMaster Osteoarthritis Index (scored 0–100, where 100 indicates worst symptoms).

the mean ± SEM WOMAC pain score was 32.7 ± 0.9 in the vitamin D group and 34.6 ± 0.9 in the placebo group. There were no significant differences in WOMAC pain score between the vitamin D group and the placebo group at any of the time points or as a trend over time (*P* for interaction with time = 0.41) (Figure 1). For both WOMAC function and stiffness, we found no significant differences between vitamin D and placebo at any of the time points. There were no significant changes in either WOMAC function or stiffness between the vitamin D group and placebo group over time (all *P* for interaction with time >0.05). Among men, while WOMAC pain did not differ between the active vitamin D group and the vitamin D placebo group at baseline (mean score 32.3 versus 33.1), the mean ± SEM WOMAC pain score at last follow-up was 29.4 ± 1.5 among men randomized to receive active vitamin D versus 34.0 ± 1.5 among those randomized to receive placebo (*P* = 0.04). The overall linear time-by-treatment interaction after adjustment for age and the other treatment group was significant (*P* = 0.04). However, the 3-way interaction (time-by-treatment-by-sex) was not significant, indicating that sex did not modify the effect of vitamin D on the WOMAC pain score. No modification of the effect of vitamin D on the WOMAC pain score was found in any of the other subgroups (Supplementary Table 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41416/abstract).

Omega-3 fatty acid. In the n-3 FA group, the mean ± SEM WOMAC pain score at baseline was 36.5 ± 0.7 in the active treatment group versus 35.4 ± 0.7 in the placebo group after adjustment for age and sex. At the time of last follow-up, the mean ± SEM WOMAC pain score was 33.6 ± 0.9 in the active n-3 FA group and 33.7 ± 0.9 in the n-3 FA placebo group. There were no significant differences between the active n-3 FA group and the n-3 FA placebo group at any of the time points or as a trend over time between the active n-3 FA and placebo groups (*P* for interaction with time = 0.77) (Figure 1). For both WOMAC function and stiffness, we found no significant differences between the active n-3 FA group and the n-3 FA placebo group at any of the time points. Neither WOMAC function nor stiffness changed significantly over time by treatment group (all *P* for interaction with time >0.05). We found no modification of the effect of n-3 FA on WOMAC pain in any of the subgroups (Supplementary Table 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.41416/abstract).

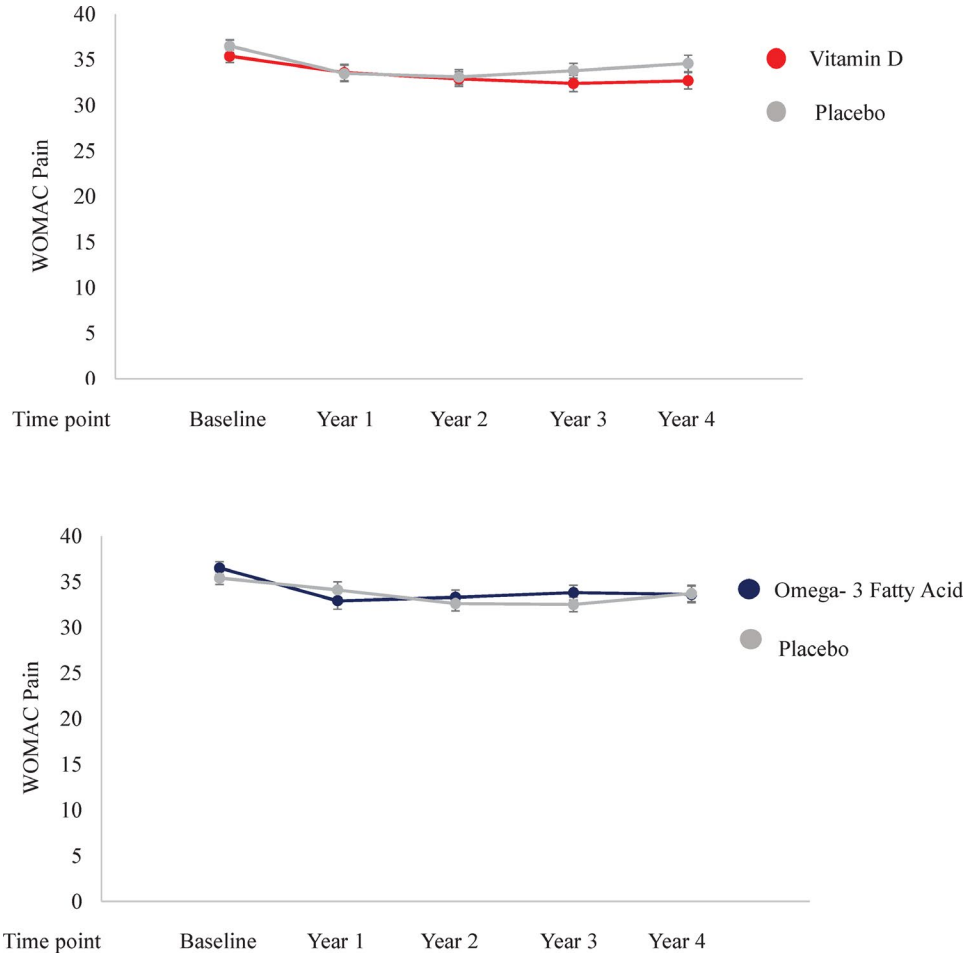

Figure 1. Repeated-measures analyses of mean Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score over time in patients receiving active vitamin D versus those receiving vitamin D placebo (top) and in patients receiving active omega-3 fatty acids (n-3 FA) versus those receiving n-3 FA placebo, with censoring for total knee replacement. Analyses were adjusted for age, sex, and other treatment group. Values are the mean ± SD.

Sensitivity analysis. The results of the 2 sensitivity analyses, one that included only the 1,221 participants who completed baseline knee pain questionnaires, and one that consisted of imputing the last pain score prior to TKR plus 10 points, did not change the results of the primary analyses. There were no significant differences in WOMAC pain score at any time point for either vitamin D or n-3 FA, and the overall linear time-by-treatment interactions were null.

Secondary outcome measures. In analyses for the secondary outcome measure of incident TKR, over a mean ± SD of 4.5 ± 1.6 person-years, the hazard ratios (HRs) for incident TKR in the vitamin D group and the n-3 FA group were 0.97 (95% confidence interval [95% CI] 0.78–1.22) and 0.99 (95% CI 0.79–1.24), respectively, compared to their respective placebo groups (vitamin D placebo and n-3 FA placebo) after adjustment for age and sex.

At baseline, 173 participants (32%) randomized to receive vitamin D reported taking occasional or daily analgesics including opioids, while 364 participants (68%) reported no opioid use. In the group randomized to receive vitamin D placebo, 170 (30%) reported occasional/daily analgesic use, and 398 (70%) reported no analgesic use. Of those in the n-3 FA group, 163 (31%) reported occasional/daily analgesic use, and 371 (69%) reported no use. In the n-3 FA placebo group, 180 participants (32%) reported occasional/daily use, and 391 (68%) reported no analgesic use. Neither vitamin D nor n-3 FA altered the use of analgesics, including opioids, over the study period (vitamin D, *P* for interaction = 0.81; n-3 FA, *P* for interaction = 0.25).

DISCUSSION

In this large, population-based cohort of patients with chronic, frequent knee pain, we found that randomized supplementation with neither vitamin D nor n-3 FA led to a decrease in patient-reported knee pain compared to placebo. Over a mean of 5.3 years of follow-up, a small decrease in reported knee pain was observed, but this occurred in both the treatment and placebo groups and may reflect regression to the mean and some

loss of participants to TKR. At no time point did pain scores differ significantly between treatment and placebo, and the treatment group did not significantly affect pain scores over time. None of the observed differences in the primary analysis exceeded an effect size of 0.1 and are therefore unlikely to have clinical implications. No clinically or statistically significant reductions in WOMAC function or stiffness were identified in either treatment group compared to the placebo group.

We saw a nominally significant effect of vitamin D on WOMAC pain score in men over time. However, the WOMAC pain score differed only at the time of last follow-up, at which point men receiving active vitamin D had a WOMAC pain score 4.5 points lower than those receiving vitamin D placebo. Overall, the interaction with sex was not significant and furthermore, as these analyses were not adjusted for multiple comparisons, this difference is unlikely to be clinically meaningful. Subgroup analyses by race, BMI, baseline fish consumption, randomization status to other treatment, baseline vitamin D level, and baseline n-3 FA index did not modify the effect of either n-3 FA or vitamin D on the WOMAC pain score over time.

Vitamin D and n-3 FA did not lead to a reduction in TKR, an indicator of severely symptomatic knee OA, compared to placebo. Additionally, we did not observe an alteration in the use of analgesics, including opioids, in groups receiving either vitamin D or n-3 FA supplementation.

Prior RCTs have demonstrated conflicting results concerning the efficacy of vitamin D on knee pain in OA. In a 24-month trial of participants with symptomatic knee OA and low vitamin D levels (12.5–60 nmoles/liter) randomized to receive vitamin D versus placebo, there were no significant changes in WOMAC pain scores between the treatment groups (12). However, post hoc analysis revealed significant improvements in the vitamin D group for total WOMAC score and WOMAC function as well as a greater proportion of Outcome Measures in Rheumatology–Osteoarthritis Research Society International responders (28). In a 12-month pilot trial of participants with symptomatic knee OA and vitamin D insufficiency randomized to receive vitamin D versus placebo, Sanghi et al reported significant improvement in WOMAC pain and function in the vitamin D group, though these differences did not meet the threshold for minimum clinically important difference (16).

A randomized trial of vitamin D and placebo in 146 patients with symptomatic OA did not find significant differences in knee pain between treatment groups, as measured by WOMAC over 24 months, though the authors raised concerns about the possibility of a Type II error (15). In a subsequent and larger 3-year trial of 474 participants in the UK with symptomatic knee OA, 50% of whom were classified as vitamin D deficient, again no significant differences between vitamin D and placebo in WOMAC pain, function, or stiffness over the study period were found, despite reducing the number of participants with vitamin D deficiency by 80% (17). Our trial adds to this body of evidence through the use of a large, racially diverse, population-based cohort with >4 years of follow-up. Our findings further support the notion that vitamin D supplements do not play a role in the reduction of knee pain in patients with OA, even among the subgroup with low vitamin D levels.

Findings from the Multicenter Osteoarthritis Study have suggested that higher systemic levels of total n-3 FA supplements were associated with less cartilage loss in the patellofemoral compartment (29). Conversely, high levels of arachidonic acid, an n-6 FA considered to be proinflammatory, were associated with synovitis (29). Randomized trials have suggested a benefit of n-3 FA in patients with rheumatoid arthritis (30,31). Trials using n-3 FA to treat OA have been limited. Hill et al compared high-dose (4.5 gm EPA + DHA) versus low-dose (0.45 gm EPA + DHA) fish oil supplementation in patients with symptomatic knee OA over 24 months, and reported improved WOMAC pain and function scores in the low-dose fish oil group (18). However, the low dose fish oil supplement also contained sunola oil, raising concern that perhaps this supplement conferred some benefit on knee OA symptoms (18). The current larger RCT allowed for comparison of n-3 FA acids to placebo and found that n-3 FA supplementation in knee OA did not confer symptomatic benefit.

Our study does have some limitations. Several knee pain cohort participants returned the baseline questionnaire later and thus did not contribute to prerandomization data. Their follow-up data contributed to our repeated measures model. Sensitivity analyses that did not include these subjects had similar findings to the primary analyses. Though the trial included fewer participants who met the definition of knee pain than anticipated in the initial power calculations, the sample size provided ample statistical power to detect differences in WOMAC pain score. We did not have knee radiographic data to assess OA severity; therefore, we are unable to assess whether vitamin D or n-3 FA supplements may have a greater role in patients with more or less severe radiographic OA findings.

Diagnosis of knee pain was based on patient report, which may be subject to bias; however, any bias should not differ by randomized treatment group. Moreover, in the subgroup of participants for whom medical records were requested and reviewed, the vast majority (92% of those whose records were reviewed) were confirmed to have knee OA. As we required at least a year of knee symptoms for eligibility, these results do not preclude the possibility that vitamin D or n-3 FA may play a role in pain management in early disease. We queried the use of opioids and/or neuropathic pain medications with a single question, and thus cannot report on whether there was a reduction in the use of opioids or in the use of neuropathic pain medications individually. The doses of vitamin D and n-3 FA used were chosen because they represented the safest and most effective doses in the parent trial. We cannot rule out the possibility that using different dosing regimens or supplement preparation may have changed our findings. This is a large, community-based cohort with efforts to ensure inclusion

of racially diverse participants, increasing the generalizability of our findings. Participants were followed up for 5 years, providing ample time to assess for changes in symptoms related to OA, which is often a slowly progressive disease.

In summary, this trial showed that neither vitamin D nor n-3 FA was associated with improvement in knee symptoms compared to placebo in older adults with chronic daily knee pain and self-reported knee OA. These results are consistent with the findings of previous smaller RCTs and suggest that supplementation with vitamin D or n-3 FA does not play a role in the management of symptomatic knee pain due to OA.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. MacFarlane had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. MacFarlane, Cook, Lee, Iversen, Gordon, Buring, Katz, Manson, Costenbader.

Acquisition of data. Cook, Gordon, Buring, Manson.

Analysis and interpretation of data. MacFarlane, Cook, Kim, Lee, Iversen, Gordon, Buring, Katz, Manson, Costenbader.

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