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Effect of Vitamin D and Omega-3 Fatty Acid Supplementation on Kidney Function in Patients With Type 2 Diabetes
A Randomized Clinical Trial

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IMPORTANCE Chronic kidney disease (CKD) is a common complication of type 2 diabetes that can lead to end-stage kidney disease and is associated with high cardiovascular risk. Few treatments are available to prevent CKD in type 2 diabetes.

OBJECTIVE To test whether supplementation with vitamin D_3 or omega-3 fatty acids prevents development or progression of CKD in type 2 diabetes.

DESIGN, SETTING, AND PARTICIPANTS Randomized clinical trial with a 2 × 2 factorial design conducted among 1312 adults with type 2 diabetes recruited between November 2011 and March 2014 from all 50 US states as an ancillary study to the Vitamin D and Omega-3 Trial (VITAL), coordinated by a single center in Massachusetts. Follow-up was completed in December 2017.

INTERVENTIONS Participants were randomized to receive vitamin D_3 (2000 IU/d) and omega-3 fatty acids (eicosapentaenoic acid and docosahexaenoic acid; 1 g/d) (n = 370), vitamin D_3 and placebo (n = 333), placebo and omega-3 fatty acids (n = 289), or 2 placebos (n = 320) for 5 years.

MAIN OUTCOMES AND MEASURES The primary outcome was change in glomerular filtration rate estimated from serum creatinine and cystatin C (eGFR) from baseline to year 5.

RESULTS Among 1312 participants randomized (mean age, 67.6 years; 46% women; 31% of racial or ethnic minority), 934 (71%) completed the study. Baseline mean eGFR was 85.8 (SD, 22.1) mL/min/1.73 m^2. Mean change in eGFR from baseline to year 5 was −12.3 (95% CI, −13.4 to −11.2) mL/min/1.73 m^2 with vitamin D_3 vs −13.1 (95% CI, −14.2 to −11.9) mL/min/1.73 m^2 with placebo (difference, 0.9 [95% CI, −0.7 to 2.5] mL/min/1.73 m^2). Mean change in eGFR was −12.2 (95% CI, −13.3 to −11.1) mL/min/1.73 m^2 with omega-3 fatty acids vs −13.1 (95% CI, −14.2 to −12.0) mL/min/1.73 m^2 with placebo (difference, 0.9 [95% CI, −0.7 to 2.6] mL/min/1.73 m^2). There was no significant interaction between the 2 interventions. Kidney stones occurred among 58 participants (n = 32 receiving vitamin D_3 and n = 26 receiving placebo) and gastrointestinal bleeding among 45 (n = 28 receiving omega-3 fatty acids and n = 17 receiving placebo).

CONCLUSIONS AND RELEVANCE Among adults with type 2 diabetes, supplementation with vitamin D_3 or omega-3 fatty acids, compared with placebo, resulted in no significant difference in change in eGFR at 5 years. The findings do not support the use of vitamin D or omega-3 fatty acid supplementation for preserving kidney function in patients with type 2 diabetes.

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Supplemental content

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Among individuals with type 2 diabetes, chronic kidney disease (CKD) is common and associated with poor health outcomes.[1] In this population, the prevalence of CKD (defined as persistently reduced glomerular filtration rate [GFR] or elevated urinary albumin excretion) is more than 25%, increases with duration of diabetes, and has remained relatively stable despite advances in diabetes care.[2-4] Type 2 diabetes is the leading cause of end-stage kidney disease (ESKD) requiring dialysis or kidney transplant in the United States, and among patients with diabetes, CKD is associated with markedly increased risks of cardiovascular events and mortality.[5]

Vitamin D and omega-3 fatty acid supplements are interventions that may have the potential to prevent the development and progression of CKD in type 2 diabetes.[6] In animal experimental models, 1,25-dihydroxyvitamin D_3 (the active hormone generated from vitamin D_3) and its analogues suppress the renin-angiotensin system, reduce kidney inflammation and fibrosis, exert prosurvival effects on podocytes, and reduce albuminuria and glomerulosclerosis,[7,8] while omega-3 fatty acids have anti-inflammatory, antithrombotic, and vascular properties that may help prevent CKD.[9] In humans, lower circulating concentrations of 25-hydroxyvitamin D (25[OH]D, used to assess vitamin D sufficiency), lower dietary intake of fish (an important source of omega-3 fatty acids), and lower plasma polyunsaturated omega-3 fatty acid concentrations have been associated with increased risk of albuminuria and GFR decline in many studies.[9-12]

However, clinical trials evaluating the kidney effects of vitamin D and omega-3 fatty acid supplements have been of short duration, evaluated only urine albumin excretion as an outcome, or examined kidney outcomes as secondary post hoc analyses.[12-15] Therefore, the Vitamin D and Omega-3 Trial to Prevent and Treat Diabetic Kidney Disease (VITAL-DKD) was conducted to assess the efficacy and safety of vitamin D and omega-3 fatty acids for prevention and treatment of CKD in patients with type 2 diabetes.

Key Points

Question In adults with type 2 diabetes, do vitamin D or omega-3 fatty acid supplements help prevent development or progression of kidney disease?

Findings In this 2 × 2 factorial randomized clinical trial that included 1312 participants with type 2 diabetes, there was no significant difference in the change in estimated glomerular filtration rate at 5 years with vitamin D_3 supplementation vs placebo (−12.3 vs −13.1 mL/min/1.73 m^2) or with omega-3 fatty acid supplementation vs placebo (−12.2 vs −13.1 mL/min/1.73 m^2).

Meaning These findings do not support the use of vitamin D or omega-3 fatty acid supplementation for preserving kidney function in adults with type 2 diabetes.

Methods

Study Design

This study was designed as an ancillary study to the Vitamin D and Omega-3 Trial (VITAL).[6] The parent trial was a randomized, double-blind, placebo-controlled trial of vitamin D and marine omega-3 fatty acids for primary prevention of cardiovascular disease and cancer (N = 25 871).[16] A single site (Brigham and Women's Hospital Department of Preventive Medicine, Boston, Massachusetts) enrolled participants from throughout the United States through mail and telephone contacts, dispensed study medications by mail, and ascertained outcomes remotely. Participants were enrolled from 50 states. Baseline blood samples were collected by the parent trial for approximately two-thirds of participants, with follow-up blood samples and urine samples collected only among a subset of generally healthy participants living in Boston.

For this study, we enrolled a subset of parent trial participants with type 2 diabetes at baseline to ascertain CKD outcomes (trial protocol available in Supplement 1). The study was approved by the Partners Human Research Committee. Participants provided written informed consent and received a stipend.

Study Population

The parent trial enrolled men aged 50 years or older and women aged 55 years or older without clinically apparent cardiovascular disease or cancer.[16] For this study, we recruited potential participants who additionally reported a physician diagnosis of diabetes at baseline and agreed to provide blood and urine samples.[6] We contacted consecutive potentially eligible individuals during the parent trial's placebo run-in phase until the enrollment goal of 1320 participants was met. We excluded those who reported a diagnosis of diabetes only during pregnancy, a diagnosis of diabetes prior to age 30 years treated with insulin for more than 20 years, or a known cause of CKD other than diabetes.

Interventions

As part of the parent trial, participants were randomly assigned to 1 of 4 treatment groups in a 2 × 2 factorial design: (1) vitamin D plus omega-3 fatty acids; (2) vitamin D plus placebo omega-3 fatty acids; (3) omega-3 fatty acids plus placebo vitamin D; or (4) both placebos.[16] Vitamin D_3 (cholecalciferol, 2000 IU) and matching inert placebo were provided by Pharmavite LLC. Omega-3 fatty acids (fish oil, 1-g capsules containing 465 mg of eicosapentaenoic acid [EPA] plus 375 mg of docosahexaenoic acid [DHA]) and matching inert placebo were provided by ProNova. Participants were asked to limit vitamin D intake from all supplemental sources combined to no more than 800 IU/d and to forgo use of nonstudy fish oil supplements. Randomization occurred from November 2011, through March 2014. Assignments were computer generated in blocks of 8 stratified by age, sex, and race. Treatment assignments were concealed to both participants and investigators.

Outcomes

The primary outcome of this study was change in estimated GFR (eGFR) from baseline to study year 5. The original primary outcome was change in albuminuria but was modified when the study duration was extended to 5 years in 2016, allowing sufficient time to assess meaningful differences in eGFR.[6]

Prespecified secondary outcomes included time to the composite outcome of at least a 40% decrease in eGFR from baseline, kidney failure, or death; time to at least a 40% decrease in eGFR from baseline;[17] and change in urine albumin-creatinine ratio (ACR) from baseline to study year 5 (analyzed as a continuous variable and as time to doubling of urine ACR to a final urine ACR ≥30 mg/g[18]). Post hoc analyses examined time to a composite outcome of at least a 40% decrease in eGFR from baseline or kidney failure as well as outcomes using at least a 30% decline in eGFR instead of at least a 40% decline in eGFR. Kidney failure was ascertained yearly by questionnaire. Urine was collected from first morning voids, and albumin and creatinine were measured on a Beckman DXC chemistry analyzer.[6]

Blood and urine samples were collected by mail prior to randomization (baseline), 2 years after randomization, and 5 years after randomization.[6] Serum creatinine and cystatin C were measured using a modified Jaffe reaction and immunoturbidimetric assay, respectively. Creatinine results are traceable to isotope dilution mass spectrometry. A shift in cystatin C values was identified with change in calibrator lot. Cystatin C concentrations were therefore harmonized across the study and to the European Reference Materials (ERM-DA471/IFCC) using a prospective quality control plan,[6] resulting in slightly different values than those previously reported (eAppendix in Supplement 2).[6] We calculated eGFR from the serum concentrations of creatinine and cystatin C using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[19]

Covariates

Baseline demographics, duration of diabetes, comorbidities, smoking and alcohol use, weight, and height were ascertained by self-report. Race and ethnicity were self-reported by participants using fixed categories to ensure that a diverse population was enrolled and to facilitate exploring racial and ethnic heterogeneity in treatment effects. Body mass index was calculated as weight divided by height squared. Medication use at baseline and follow-up was ascertained using a detailed questionnaire that listed all glucose- and blood pressure–lowering medications available at the time of administration, updated throughout the study. Adherence to study medications was assessed by self-report, with a subset of highly adherent participants defined as reporting use of study medications at least two-thirds of the time. At baseline and year 2, serum 25(OH)D concentrations and the plasma omega-3 index (EPA plus DHA as a percentage of total fatty acids) were measured by liquid chromatography–tandem mass spectrometry. C-reactive protein was measured on a Beckman DXC chemistry analyzer.

Sample Size

A sample of 1320 participants provided 80% power to detect a difference in change in eGFR from baseline to end of study of at least 2.3 mL/min/1.73 m^2, comparing each active treatment with placebo, at a 2-sided $\alpha = .05$, assuming distributions of change in eGFR observed in preliminary data and 80% of participants returning follow-up samples (see analysis plan in Supplement 1).[6] A difference in change of at least 2.3 mL/min/1.73 m^2 over 5 years was judged by investigators to include the smallest differences of clinical relevance and captures changes more recently suggested as a surrogate outcome for ESKD (≥0.75 mL/min/1.73 m^2 per year, equivalent to ≥3.75 mL/min/1.73 m^2 over 5 years).[20,21]

Data Analysis

We used linear mixed models to summarize changes in eGFR and urine ACR over time and to test whether these changes differed by treatment assignment. Random intercepts were included in the linear mixed models to account for the correlation within participant. Time was modeled as 3 nonordered variables (baseline, year 2, and year 5), with interaction terms included for treatment × time. Terms for linear age, sex, and their interactions with time were also included. Models with eGFR as the outcome additionally adjusted for baseline urine ACR and its interaction with time. The *P* value for interaction of treatment with time (year 5) was used to test treatment effects. A 2-tailed $P < .05$ was considered significant for each intervention. Because of the potential for type I error due to multiple comparisons, findings for analyses of secondary end points should be interpreted as exploratory.

For primary analyses, all participants were analyzed according to their randomized treatment group, regardless of adherence or follow-up (full analytic population). Multiple imputation (M = 20) was used for missing data, including missing data for follow-up outcome measurements, to minimize potential bias due to loss to follow-up; estimates were combined across imputations using Rubin rules.[22] Secondary analyses were restricted to participants who returned the relevant biosamples at baseline and year 5 (complete case population), were restricted to those who reported high adherence to study medications, or excluded participants who reported symptoms of possible urinary tract infection at the time of urine collection (for analyses of change in urine albumin excretion only).

For the vitamin D intervention, prespecified subgroup analyses were defined based on race and ethnicity and baseline 25(OH)D concentrations, urine ACR, and eGFR[6]; body mass index was added when the parent trial reported interaction by body mass index with regard to incident cancer.[23] For the omega-3 fatty acid intervention, prespecified subgroup analyses were defined based on baseline omega-3 index and C-reactive protein[6]; dietary fish intake was added when the parent trial reported interaction by dietary fish intake with regard to cardiovascular events.[24] For each of these subgroups, we added linear terms for the subgroup, subgroup × time interaction, and subgroup × time × treatment interaction to the model used in primary analyses; a Wald test of the subgroup × time × treatment coefficient tested whether there was a significant interaction in treatment effect. We examined secondary categorical outcomes using Cox proportional hazards models and tested the proportional hazards assumption using Schoenfeld residuals.

All analyses were conducted using the R version 3.6.0 computing environment (R Foundation for Statistical Computing).

Results

Participant Characteristics

At baseline, participants enrolled in this study had a mean age of 67.6 years and a median duration of diagnosed diabetes of 6 to 10 years (**Table 1**); 46% were women, and 31% were of racial or ethnic minority. Biguanides, followed by sulfonylureas, were the most commonly used glucose-lowering medications, with insulin use reported by 20%. Less than 10% of participants reported using a dipeptidyl peptidase 4 inhibitor or glucagon-like peptide 1 receptor agonist, and sodium-glucose cotransporter 2 inhibitors were not yet commercially available. Antihypertensive medications were used by 80% of participants, including 61% using a renin-angiotensin system inhibitor. Mean baseline eGFR was 85.8 mL/min/1.73 m^2, and eGFR was less than 60 mL/min/1.73 m^2 for 165 participants (13%). Urine ACR was at least 30 mg/g for 117 participants (9%), including 24 (2%) with an ACR of at least 300 mg/g.

Retention and Adherence

At least 1 follow-up blood sample was provided by 1090 (83%) of the 1312 participants who were randomized, including 934 (71% of randomized participants; 76% of those alive) 5 years after randomization (**Figure 1**). At least 1 follow-up urine sample was provided by 1091 participants (83%), including 945 (72% of randomized participants; 77% of those alive) 5 years after randomization.

Adherence to at least two-thirds of study medications was reported by 92% and 88% of participants at 2 and 5 years, respectively, after randomization for vitamin D (or matching placebo) and by 91% and 89%, respectively, at 2 and 5 years for omega-3 fatty acids (or matching placebo) (eTable 1 in Supplement 2). Two years after randomization, mean serum 25(OH)D concentrations were 41.4 (SD, 11.0) ng/mL for participants assigned to vitamin D and 29.8 (SD, 11.1) ng/mL for participants assigned to vitamin D placebo ($P < .001$). Two years after randomization, mean omega-3 indexes were 3.6% (SD, 1.0%) for participants assigned to omega-3 fatty acids and 2.3% (SD, 0.8%) for participants assigned to omega-3 fatty acid placebo ($P < .001$).

Regardless of treatment assignment, proportions of participants using biguanides, sulfonylureas, insulin, and angiotensin-converting enzyme inhibitors remained relatively stable over the course of the study (eTables 2 and 3 in Supplement 2). Use of dipeptidyl peptidase 4 inhibitors (9% at baseline and 14% at year 5) and angiotensin II receptor blockers (20% at baseline and 29% at year 5) increased modestly, and 5% of participants initiated a sodium-glucose cotransporter 2 inhibitor, with similar changes according to treatment assignment.

Primary Outcome: Change in eGFR

Mean eGFR was 85.8 (SD, 22.1) mL/min/1.73 m^2 at baseline, 80.0 (SD, 21.5) mL/min/1.73 m^2 at year 2, and 73.5 (SD, 21.9) mL/min/1.73 m^2 at year 5 (**Figure 2**). Mean change in eGFR from baseline to year 5 was −12.7 (95% CI, −13.6 to −11.7) mL/min/1.73 m^2 in the full analytic population and −12.4 (95% CI, −13.3 to −11.4) mL/min/1.73 m^2 among the 932 participants with eGFR data at both baseline and year 5. Mean change in eGFR from baseline to year 5 was −12.3 (95% CI, −13.4 to −11.2) mL/min/1.73 m^2 with vitamin D_3 vs −13.1 (95% CI, −14.2 to −11.9) mL/min/1.73 m^2 with placebo. Mean change in eGFR was −12.2 (95% CI, −13.3 to −11.1) mL/min/1.73 m^2 with omega-3 fatty acids vs −13.1 (95% CI, −14.2 to −12.0) mL/min/1.73 m^2 with placebo. At year 5, there was no significant difference in change in eGFR according to treatment (0.9 [95% CI, −0.7 to 2.5] mL/min/1.73 m^2 comparing vitamin D with placebo; 0.9 [95% CI, −0.7 to 2.6] mL/min/1.73 m^2 comparing omega-3 fatty acids with placebo) (**Table 2**), and there was no significant interaction between treatment assignments ($P = .42$).

Similar results were observed when analyses were restricted to participants who provided serum samples at baseline and year 5 or to participants who reported consistent high adherence to study medications (eTables 4 and 5 in Supplement 2). No significant subgroup heterogeneity was observed for the effect of either vitamin D or omega-3 fatty acids on change in eGFR (**Figure 3** and **Figure 4**). Neither change in serum 25(OH)D nor change in omega-3 index from baseline to year 2 significantly correlated with change in eGFR from baseline to year 5 (eFigure 1 in Supplement 2).

Secondary Outcomes

Of 3 prespecified secondary outcomes, none differed significantly by treatment assignment for either intervention. The composite outcome of at least a 40% decline in eGFR, kidney failure, or death occurred in 164 participants (80 with measured decline in eGFR ≥40%, 11 reporting kidney failure, and 80 deaths, with some participants meeting multiple components) and did not differ significantly by treatment assignment (hazard ratio, 0.92 [95% CI, 0.68-1.25] comparing vitamin D with placebo; hazard ratio, 1.11 [95% CI, 0.81-1.50] comparing omega-3 fatty acids with placebo). The secondary outcome of at least 40% decline in eGFR also did not differ significantly by treatment assignment (**Table 3**).

The geometric mean urine ACR was 5.1 mg/g (95% CI, 4.6-5.7 mg/g) at year 2 and 9.2 mg/g (95% CI, 8.4-10.1 mg/g) at year 5 (Figure 2). Urine ACR increased approximately 3-fold from baseline to year 5, but there was no significant difference in change in urine ACR according to assignment to vitamin D or placebo or to omega-3 fatty acids or placebo (Table 3 and eTable 6 in Supplement 2). Similar results were observed in relevant subgroups, when analyses were restricted to participants who provided urine samples at baseline and year 5, when analyses were restricted to those reporting consistent adherence to study medications, and when participants who reported symptoms of possible urinary tract infection at the time of urine collection were excluded (eFigures 2 and 3 and eTables 7, 8, and 9 in Supplement 2).

Exploratory and Post Hoc Outcomes

A composite outcome of at least a 40% eGFR decline or kidney failure as well as composite outcomes including an eGFR decline of at least 30% were not prespecified and did not differ significantly according to treatment assignment (eTable 10

Table 1. Baseline Characteristics of Participants in the Vitamin D and Omega-3 Trial to Prevent and Treat Diabetic Kidney Disease[a]

Characteristics	Treatment Group, No. (%)			
	Vitamin D_3 and Omega-3 Fatty Acids (n = 370)	Vitamin D_3 and Placebo (n = 333)	Omega-3 Fatty Acids and Placebo (n = 289)	Two Placebos (n = 320)
Demographics				
Sex				
Female	184 (50)	143 (43)	131 (45)	151 (47)
Male	186 (50)	190 (57)	158 (55)	169 (53)
Age, mean (SD), y	67.4 (7.3)	67.4 (6.7)	68.2 (6.7)	67.5 (6.9)
Race/ethnicity	361	327	284	314
Non-Hispanic white	240 (66)	211 (65)	199 (70)	207 (66)
Non-Hispanic black	73 (20)	75 (23)	59 (21)	71 (23)
Hispanic	20 (6)	21 (6)	16 (6)	18 (6)
Asian/Pacific Islander	13 (4)	8 (2)	7 (2)	7 (2)
American Indian/Alaska Native	6 (2)	4 (1)	1 (<1)	2 (1)
Other	9 (2)	8 (2)	2 (1)	9 (3)
More than high school education	303 (82)	283 (85)	243 (84)	270 (85)
Medical history and lifestyle				
Duration of diabetes, y				
<1	13 (4)	15 (5)	5 (2)	9 (3)
1-2	52 (14)	43 (13)	45 (16)	37 (12)
3-5	78 (21)	72 (22)	62 (22)	78 (24)
6-10	107 (29)	83 (25)	74 (26)	96 (30)
11-20	90 (24)	82 (25)	69 (24)	74 (23)
>20	29 (8)	37 (11)	33 (11)	25 (8)
Current smoking	22 (6)	17 (5)	15 (5)	25 (8)
Current alcohol use	191 (53)	184 (56)	151 (53)	168 (54)
Medication and supplement use at randomization				
Glucose-lowering medications				
Biguanides	247 (67)	222 (67)	199 (69)	221 (69)
Sulfonylureas	109 (29)	100 (30)	85 (29)	99 (31)
Insulin	67 (18)	68 (20)	57 (20)	66 (21)
Thiazolidinediones	32 (9)	32 (10)	24 (8)	36 (11)
Dipeptidyl peptidase 4 inhibitors	33 (9)	26 (8)	22 (8)	34 (11)
Glucagon-like peptide 1 receptor agonists	9 (2)	14 (4)	16 (6)	9 (3)
Antihypertensive medications	293 (80)	263 (80)	231 (81)	258 (81)
No. of classes, mean (SD)	1.4 (1.2)	1.4 (1.1)	1.4 (1.1)	1.4 (1.2)
ACE inhibitors or ARBs	223 (60)	205 (62)	177 (61)	198 (62)
ACE inhibitors	163 (44)	148 (44)	121 (42)	133 (42)
Diuretics	111 (30)	87 (26)	82 (28)	84 (26)
β-Blockers	91 (25)	74 (22)	62 (21)	68 (21)
Calcium channel blockers	75 (20)	76 (23)	58 (20)	66 (21)
Angiotensin II receptor blockers	67 (18)	64 (19)	60 (21)	69 (22)
Mineralocorticoid receptor antagonists	2 (1)	3 (1)	0	4 (1)
Cholesterol-lowering medications	250 (69)	238 (73)	199 (71)	223 (71)
Supplemental vitamin D (≥800 IU/d) at randomization	146 (39)	138 (41)	124 (43)	128 (40)
Supplemental calcium (≥1200 mg/d) at randomization	84 (23)	66 (20)	81 (28)	81 (25)
Physical characteristics				
Body mass index, mean (SD)[b]	31.4 (6.5)	31.8 (6.6)	30.8 (6.6)	31.6 (7.3)
Laboratory values at baseline				
Serum creatinine, mean (SD), mg/dL	0.8 (0.3)	0.9 (0.3)	0.8 (0.2)	0.9 (0.2)
Cystatin C, mean (SD), mg/L	0.9 (0.3)	0.9 (0.3)	0.9 (0.3)	0.9 (0.3)
Urine ACR, median (IQR), mg/g	2.9 (0.5-7.6)	2.8 (0.5-8.8)	2.9 (0.5-7.1)	3.2 (1.0-6.7)
25-hydroxyvitamin D, mean (SD), ng/mL	29.6 (10.6)	29.0 (9.8)	30.2 (10.3)	30.1 (9.9)
<20	62 (17)	48 (15)	41 (15)	44 (14)
20 to <30	109 (31)	121 (38)	87 (32)	116 (37)
≥30	184 (52)	148 (47)	146 (53)	151 (49)

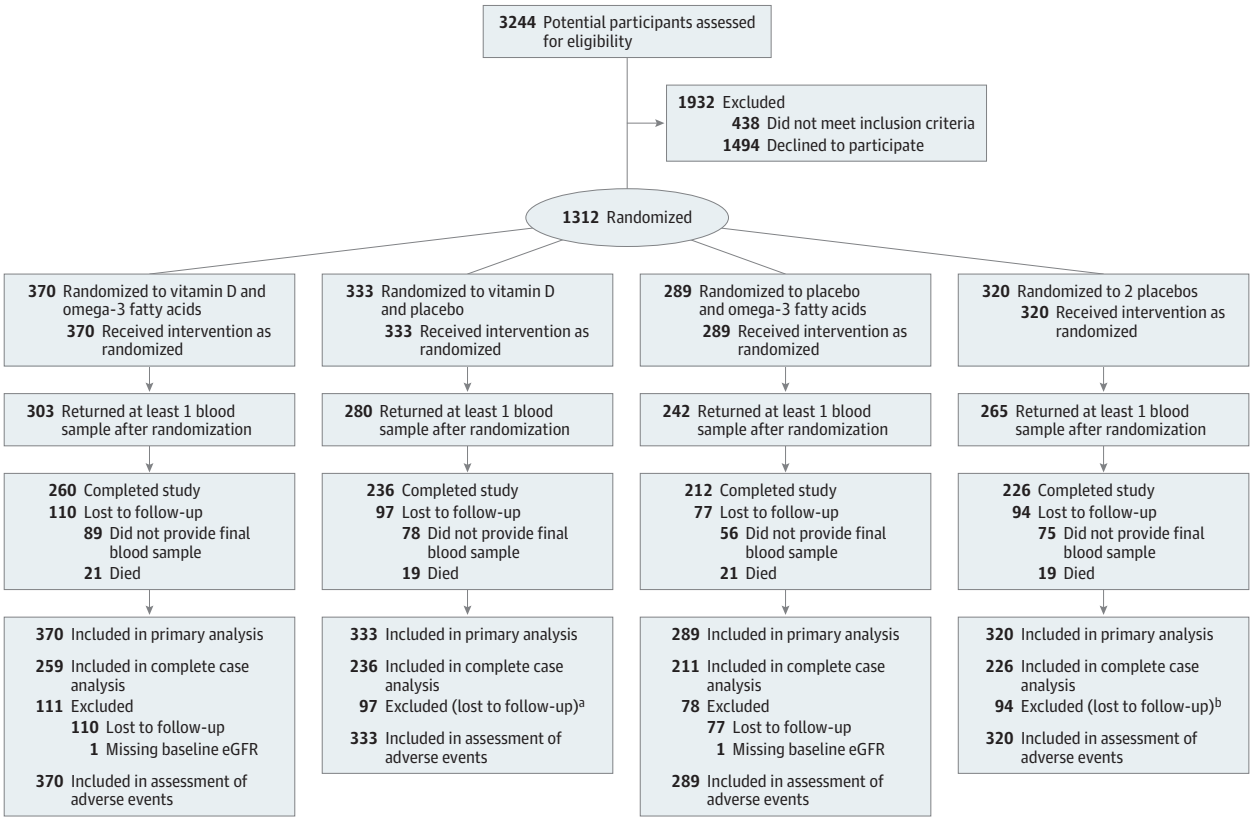
Abbreviations: ACE, angiotensin-converting enzyme; ACR, albumin-creatinine ratio; IQR, interquartile range.

SI conversion factor: To convert creatinine to µmol/L, multiply by 88.4.

[a] Data are reported as No. (%) of participants unless otherwise indicated. Percentages are calculated as percentage of nonmissing responses.

[b] Calculated as weight in kilograms divided by height in meters squared.

Figure 1. Participant Flow in the Vitamin D and Omega-3 Trial to Prevent and Treat Diabetic Kidney Disease



eGFR indicates estimated glomerular filtration rate.

[a] Of the 97 lost to follow-up, 1 was also missing baseline eGFR.

[b] Of the 94 lost to follow-up, 1 was also missing baseline eGFR.

in Supplement 2). There were no statistically significant violations of the proportional hazards assumption for any secondary outcome.

Adverse Events

Adverse events were similar comparing both vitamin D and omega-3 fatty acid supplementation with respective placebos (eTable 11 in Supplement 2). For example, kidney stones occurred among 58 participants (32 receiving vitamin D_3 and 26 receiving placebo) and gastrointestinal bleeding occurred among 45 participants (28 receiving omega-3 fatty acids and 17 receiving placebo).

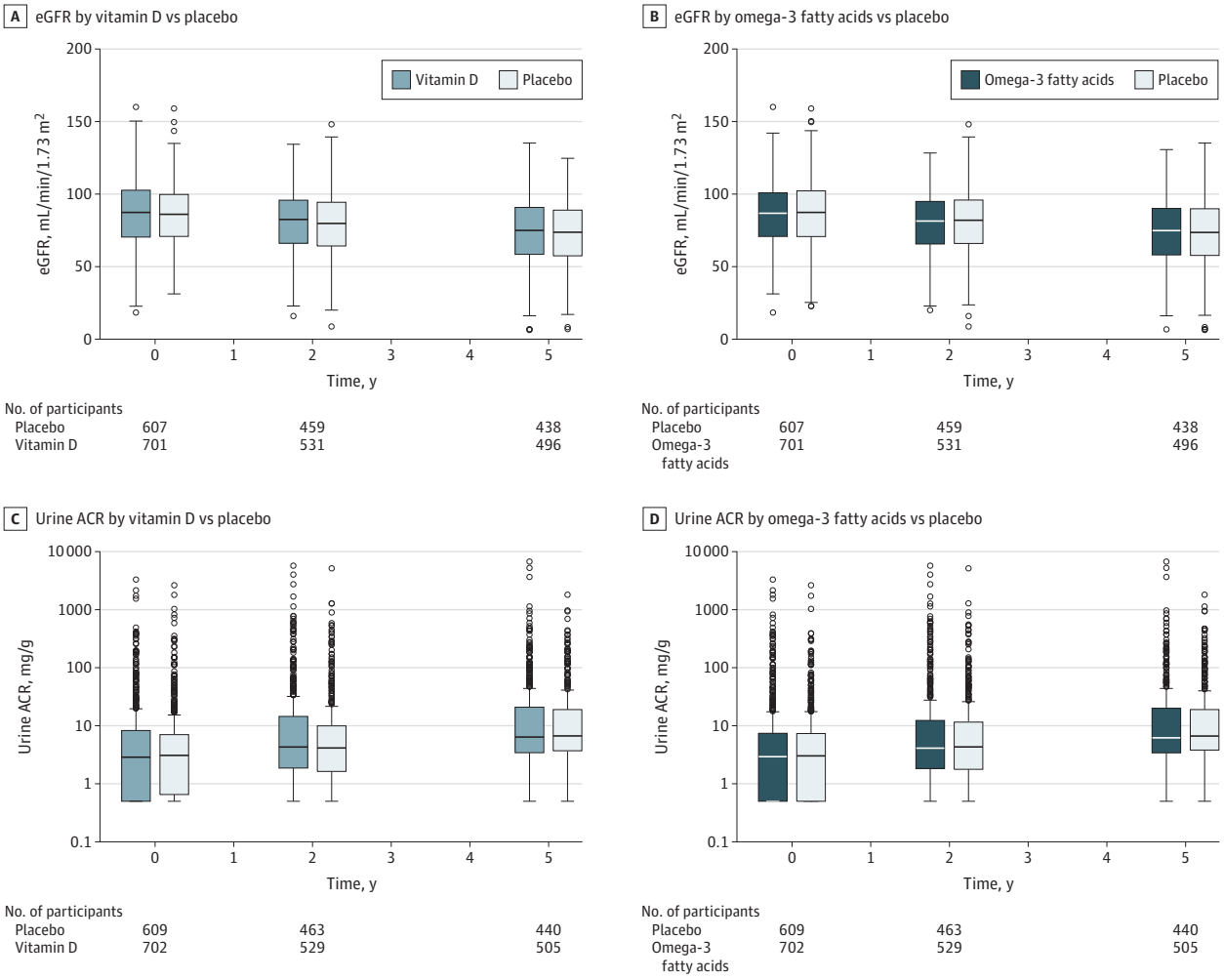
Discussion

Among adults with type 2 diabetes in this randomized clinical trial, neither vitamin D nor omega-3 fatty acid supplementation significantly slowed eGFR decline over 5 years. Results were consistent in sensitivity analyses restricted to participants with complete data or to participants who were adherent to study interventions, and secondary outcomes of large changes in eGFR and change in urine albumin excretion also showed no statistically significant differences between groups. Altogether, these results suggest that neither vitamin D nor omega-3 fatty acids have appreciable kidney benefits among the broad population of patients with diagnosed type 2 diabetes.

This study assessed change in eGFR over 5 years as the primary outcome because this is a clinically relevant outcome with which treatment effects could be assessed with high power. Progressive GFR decline leads to ESKD, and faster eGFR decline—even within the "normal range" of eGFR—is associated with increased risk of ESKD, cardiovascular events, and death.[25] Change in eGFR over 5 years is akin to eGFR slope, a primary outcome of many prior clinical trials that meets many criteria for a surrogate outcome.[20,21]

In the setting of type 2 diabetes, 5 years is sufficient time for substantial eGFR decline to occur with standard treatments. Mean eGFR in the study population decreased by 12.7 mL/min/1.73 m^2 over 5 years. This magnitude of eGFR decline is more than expected with aging alone (2-3 mL/min/1.73 m^2 over 5 years[19]) and probably reflects cumulative effects of relatively long-standing type 2 diabetes. Changes in medications over the course of the study could also affect change in eGFR, but medication changes were small in this study and similar across randomized groups. The study was powered to detect even modest differences in change in eGFR, as little as 2.3 mL/min/1.73 m^2, but neither intervention had

Figure 2. Distributions of eGFR and Urine ACR Over the Course of the Study According to Treatment Assignment



ACR indicates urine albumin-creatinine ratio; eGFR, estimated glomerular filtration rate. The center horizontal line in each box indicates the median; top and bottom box borders indicate the first and third quartiles, respectively. Whiskers depict the most extreme observation within 1.5× the interquartile range (IQR) of the nearest quartile; circles show all points lying beyond 1.5× the IQR of the nearest quartile. Urine ACR plots are shown on a log scale. Numbers shown are participants contributing data at each time point.

such an effect, and 95% confidence intervals excluded differences in eGFR decline that may be considered a reasonable surrogate outcome (0.75 mL/min/1.73 m^2 per year,[20,21] equivalent to 3.75 mL/min/1.73 m^2 over 5 years).

A composite outcome of decline in eGFR of at least 40%, development of kidney failure, or death was evaluated as a secondary outcome. There were insufficient numbers of events to effectively evaluate treatment effects on this outcome, which is conceptually similar to the primary outcome but is considered a valid surrogate outcome for ESKD.[17] Nonetheless, hazard ratios for this outcome were close to 1 for both vitamin D and omega-3 fatty acids. Urine albumin excretion, a marker of kidney damage that is complementary to eGFR, was low in the study population but did increase approximately 3-fold over the course of the study. However, mean urine albumin excretion was also not significantly affected by vitamin D or omega-3 fatty acids. Null results for secondary outcomes support the overall lack of effect for the primary outcome and suggest that neither vitamin D nor omega-3 fatty acids have kidney effects in this study population.

In a meta-analysis of short-term clinical trials, treatment with 1,25-dihydroxyvitamin D_3 or a 1,25-diydroxyvitamin D_3 analogue reduced urine albumin excretion, compared with placebo.[26] However, their long-term effects on eGFR are not known, and these treatments are associated with adverse effects such as hypercalcemia. Supplemental forms of vitamin D, such as vitamin D_3, may be more appropriate for widespread use for prevention. Prior clinical trials of vitamin D_3 assessing kidney outcomes have been small (range, 51-63 participants) and of short duration.[15,27,28] The results of this study suggest little or no effect of vitamin D_3 supplements on CKD in the setting of type 2 diabetes but do not address uncertainty regarding the long-term kidney effects of 1,25-dihydroxyvitamin D_3.

Table 2. Primary Outcome: Effects of Vitamin D and Omega-3 Fatty Acids on Change in eGFR From Baseline to Year 5

	Active Intervention			Placebo			Difference in Change From Baseline[a]	
	No.	eGFR, Mean (95% CI), mL/min/1.73 m^2	eGFR Change From Baseline, Mean (95% CI), mL/min/1.73 m^2[b]	No.	eGFR, Mean (95% CI), mL/min/1.73 m^2	eGFR Change From Baseline, Mean (95% CI), mL/min/1.73 m^2[b]	Mean Difference (95% CI), mL/min/1.73 m^2	*P* Value[c]
Vitamin D								
Baseline[d]	701	86.3 (84.6-88.0)		607	85.3 (83.7-87.0)			
Year 2	531	80.6 (78.8-82.4)	−5.2 (−6.2 to −4.2)	459	79.3 (77.3-81.2)	−6.1 (−7.1 to −5.1)	0.9 (−0.6 to 2.5)	
Year 5	496	74.3 (72.3-76.2)	−12.3 (−13.4 to −11.2)	438	72.5 (70.6-74.5)	−13.1 (−14.2 to −11.9)	0.9 (−0.7 to 2.5)	.25
Omega-3 Fatty Acids								
Baseline[d]	657	85.7 (84.1-87.3)		651	86.0 (84.2-87.8)			
Year 2	499	79.4 (77.6-81.2)	−5.7 (−6.8 to −4.7)	491	80.6 (78.6-82.5)	−5.5 (−6.5 to −4.6)	−0.3 (−1.8 to 1.3)	
Year 5	472	73.7 (71.8-75.6)	−12.2 (−13.3 to −11.1)	462	73.2 (71.1-75.3)	−13.1 (−14.2 to −12.0)	0.9 (−0.7 to 2.6)	.27

Abbreviation: eGFR, estimated glomerular filtration rate.

[a] Modeled difference, with positive values indicating higher eGFR at year 5 (slower decline in eGFR from baseline) among participants randomized to the active intervention compared with those randomized to the corresponding placebo intervention. Derived from a linear mixed model that includes adjustment for age, sex, and baseline urine albumin-creatinine ratio and accounts for missing data using multiple imputation.

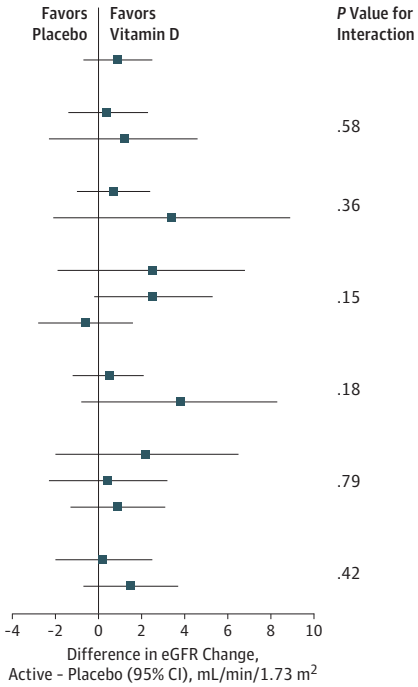
[b] Change from baseline summarized over all participants using multiple imputation.

[c] Test of the difference in change in eGFR from baseline to year 5.

[d] Four participants were missing baseline eGFR values.

Figure 3. Effects of Vitamin D vs Placebo on Change in eGFR Among Participant Subgroups

	Placebo		Vitamin D		
	No. of Participants	Mean (SD) Change in eGFR, mL/min/1.73 m^2	No. of Participants	Mean (SD) Change in eGFR, mL/min/1.73 m^2	*P* Value for Interaction
Overall	703	-13.1 (14.4)	609	-12.3 (14.8)	
Race/ethnicity					
White	451	-12.1 (13.7)	406	-11.7 (13.5)	.58
Black	148	-15.9 (16.0)	130	-14.7 (17.1)	
Baseline urine ACR, mg/g					
<30	635	-12.4 (14.2)	559	-11.8 (14.4)	.36
≥30	67	-20.4 (15.0)	50	-17.3 (17.4)	
Baseline 25-hydroxyvitamin D, ng/mL					
<20	110	-14.6 (13.9)	85	-12.2 (17.1)	
20 to <30	230	-14.7 (15.2)	203	-12.4 (14.0)	.15
≥30	332	-11.5 (13.9)	297	-12.3 (14.6)	
Baseline eGFR, mL/min/1.73 m^2					
≥60	612	-14.1 (14.4)	531	-13.7 (14.5)	.18
<60	89	-5.8 (12.4)	76	-3.0 (13.6)	
Body mass index					
<25	87	-12.9 (13.2)	87	-10.5 (11.7)	
25 to <30	228	-12.3 (14.1)	205	-12.2 (14.4)	.79
≥30	364	-13.7 (15.0	294	-12.8 (15.7)	
Omega-3 fatty acid randomization group					
Placebo	333	-13.2 (14.9)	320	-13.0 (14.7)	.42
Omega-3 fatty acids	370	-12.9 (13.9)	289	-11.7 (14.9)	

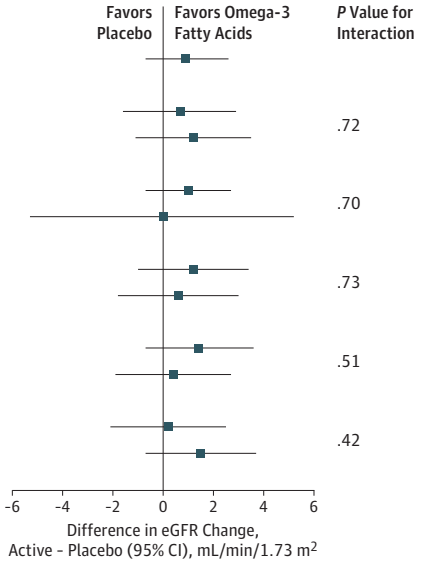


ACR indicates urine albumin-creatinine ratio; eGFR, estimated glomerular filtration rate. Body mass index is calculated as weight in kilograms divided by height in meters squared. Estimates are differences in change in eGFR from baseline to year 5 comparing active treatment with placebo, adjusted for age, sex, and baseline urine ACR.

A meta-analysis of 17 small clinical trials (a total of 626 participants with varied causes of CKD, including diabetes) suggested that omega-3 fatty acid supplementation reduced albuminuria but did not have significant effects on eGFR.[12] Two trials among those with preexisting coronary heart disease assessed the effects of omega-3 fatty acids on eGFR or albuminuria post hoc. Among 2344 older adults with myocardial infarction (19% with diabetes), EPA plus DHA added to margarine was reported to attenuate eGFR decline by 2.1 mL/min/1.73 m^2 over 3.3 years compared with placebo, although a similar effect was not observed in a treatment group that additionally received linoleic acid.[13] Among 262 participants with stable coronary artery disease, EPA plus DHA treatment for 1 year was reported to reduce urinary albumin excretion only

Figure 4. Effects of Omega-3 Fatty Acids vs Placebo on Change in eGFR Among Participant Subgroups

	Placebo		Omega-3 Fatty Acids	
	No. of Participants	Mean (SD) Change in eGFR, mL/min/1.73 m^2	No. of Participants	Mean (SD) Change in eGFR, mL/min/1.73 m^2
Overall	659	-13.1 (14.8)	653	-12.2 (14.5)
Baseline EPA/DHA, %				
≤2.2	332	-13.3 (15.0)	347	-12.5 (14.6)
>2.2	308	-12.9 (14.6)	292	-11.9 (14.4)
Baseline urine ACR, mg/g				
<30	596	-12.6 (14.8)	598	-11.5 (13.8)
≥30	62	-18.1 (14.2)	55	-19.1 (18.3)
Baseline hsCRP, mg/dL				
≤0.2	334	-12.9 (14.3)	335	-11.5 (13.4)
>0.2	323	-13.3 (15.3)	316	-13.0 (15.5)
Baseline fish intake				
<1.5 servings/wk	354	-13.4 (15.3)	329	-11.8 (14.7)
≥1.5 servings/wk	295	-12.9 (14.3)	310	-12.7 (14.3)
Vitamin D randomization group				
Placebo	289	-13.2 (14.9)	320	-12.9 (13.9)
Vitamin D	370	-13.0 (14.7)	333	-11.7 (14.9)



ACR indicates urine albumin-creatinine ratio; DHA, docosahexaenoic acid; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; hsCRP, high-sensitivity C-reactive protein. Estimates are differences in change in eGFR from baseline to year 5 comparing active treatment with placebo, adjusted for age, sex, and baseline urine ACR.

Table 3. Secondary Outcomes: Effects of Vitamin D and Omega-3 Fatty Acids on Change in eGFR and Urine Albumin Excretion[a]

	Active Intervention		Placebo				
Outcomes	No. of Events	Incidence Rate per 100 Person-Years (95% CI)	No. of Events	Incidence Rate per 100 Person-Years (95% CI)	Difference in Incidence Rate per 100 Person-Years (95% CI)	Active vs Placebo, Hazard Ratio (95% CI)[b]	*P* Value[c]
Vitamin D Intervention							
≥40% Decline in eGFR, kidney failure during study, or death	85	2.5 (2.0-3.0)	79	2.7 (2.1-3.3)	−0.2 (−1.0 to 0.6)	0.92 (0.68-1.25)	.61
≥40% Decline in eGFR	42	1.6 (1.1-2.1)	38	1.7 (1.2-2.1)	−0.1 (−0.7 to 0.6)	0.97 (0.63-1.51)	.90
Doubling in urine ACR and ≥30 mg/g	111	4.4 (3.6-5.2)	74	3.3 (2.5-4.0)	1.1 (0.0 to 2.2)	1.34 (1.00-1.80)	.05
Omega-3 Intervention							
≥40% Decline in eGFR, kidney failure during study, or death	86	2.7 (2.2-3.3)	78	2.5 (1.9-3.0)	0.3 (−0.5 to 1.1)	1.11 (0.81-1.50)	.52
≥40% Decline in eGFR	40	1.6 (1.1-2.1)	40	1.6 (1.2-2.1)	0.0 (−0.7 to 0.7)	0.99 (0.64-1.54)	.97
Doubling in urine ACR and ≥30 mg/g	96	4.0 (3.2-4.8)	89	3.7 (3.0-4.5)	0.3 (−0.8 to 1.4)	1.08 (0.81-1.44)	.60

Abbreviations: eGFR, estimated glomerular filtration rate; ACR, albumin-creatinine ratio.

[a] Prespecified secondary outcomes include time to the composite outcome of ≥40% decrease in eGFR from baseline, kidney failure, or death, time to 40% decrease in eGFR from baseline, and change in urine ACR from baseline to study year 5 (analyzed in this table as time to doubling of urine ACR to a final urine ACR ≥30 mg/g and in eTable 6 in Supplement 2 as a continuous variable).

[b] Hazard ratios are from a Cox regression model; eGFR outcomes exclude 4 participants who were missing baseline eGFR data and urine ACR outcome excludes 1 participant who was missing baseline urine ACR data.

[c] Test of the null hypothesis that hazard ratio = 1.

among the subset of participants with diabetes (n = 79), although multiple testing was not addressed.[14] These studies were exploratory in nature and were not confirmed by the results of the current study.

The results of this study apply to relatively healthy adults with type 2 diabetes. Participants with known cardiovascular disease, cancer, and ESKD were excluded from the VITAL trial.[16] Furthermore, participants were not selected based on presence or severity of CKD or on evidence of vitamin D or omega-3 fatty acid deficiency. There were few participants with these characteristics, and the trial was not powered to assess these specific participants. Point estimates from subgroup analyses among patients with evidence of CKD (eGFR <60 mL/min/1.73 m^2 or urine ACR ≥30 mg/g), serum 25(OH)D concentration less than 30 ng/mL, or normal body mass index at baseline were in favor of the vitamin D intervention, but the 95%

confidence intervals around these estimates crossed the null and tests for interaction were not statistically significant, so these observations are purely hypothesis generating, and speculation should be viewed with caution.

Strengths of this study include the rigorous randomized design, the relatively large sample size and long duration of follow-up for the interventions studied, good adherence to study medications to enhance internal validity, and consistent results in sensitivity analyses and evaluation of secondary outcomes.

Limitations

This study has several limitations. First, there were modest numbers of eGFR and urine ACR measurements collected per participant, limiting evaluation of slopes and time-to-event analyses. Second, there were insufficient numbers of events for widely accepted surrogate kidney end points, which were evaluated as secondary outcomes. Third, power was limited to assess effects among subgroups who may derive more benefit from the study interventions than the overall type 2 diabetes population. Fourth, not all participants returned a serum sample to calculate final (year 5) eGFR, but 71% did so, and 83% returned a serum sample after randomization. The primary analytic approach accounted for missing data to minimize bias, and a complete case approach yielded similar results.

Conclusions

Among adults with type 2 diabetes, supplementation with vitamin D_3 or omega-3 fatty acids, compared with placebo, resulted in no significant difference in change in eGFR at 5 years. The findings do not support the use of vitamin D or omega-3 fatty acid supplementation for preserving kidney function in patients with type 2 diabetes.

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REFERENCES

1. Tuttle KR, Bakris GL, Bilous RW, et al. Diabetic kidney disease: a report from an ADA consensus conference. *Diabetes Care*. 2014;37(10):2864-2883. doi:10.2337/dc14-1296

2. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney disease in the United States. *JAMA*. 2011;305(24):2532-2539. doi:10.1001/jama.2011.861

3. Zelnick LR, Weiss NS, Kestenbaum BR, et al. Diabetes and CKD in the United States population, 2009-2014. *Clin J Am Soc Nephrol*. 2017;12(12):1984-1990. doi:10.2215/CJN.03700417

4. Afkarian M, Zelnick LR, Hall YN, et al. Clinical manifestations of kidney disease among US adults with diabetes, 1988-2014. *JAMA*. 2016;316(6):602-610. doi:10.1001/jama.2016.10924

5. Afkarian M, Sachs MC, Kestenbaum B, et al. Kidney disease and increased mortality risk in type 2 diabetes. *J Am Soc Nephrol*. 2013;24(2):302-308. doi:10.1681/ASN.2012070718

6. de Boer IH, Zelnick LR, Lin J, et al. Vitamin D and omega-3 trial to prevent and treat diabetic kidney disease: rationale, design, and baseline characteristics. *Contemp Clin Trials*. 2018;74:11-17. doi:10.1016/j.cct.2018.09.014

7. Zhang Z, Zhang Y, Ning G, Deb DK, Kong J, Li YC. Combination therapy with AT1 blocker and vitamin D analog markedly ameliorates diabetic nephropathy: blockade of compensatory renin increase. *Proc Natl Acad Sci U S A*. 2008;105(41):15896-15901. doi:10.1073/pnas.0803751105

8. Ito I, Waku T, Aoki M, et al. A nonclassical vitamin D receptor pathway suppresses renal fibrosis. *J Clin Invest*. 2013;123(11):4579-4594. doi:10.1172/JCI67804

9. Shapiro H, Theilla M, Attal-Singer J, Singer P. Effects of polyunsaturated fatty acid consumption in diabetic nephropathy. *Nat Rev Nephrol*. 2011;7(2):110-121. doi:10.1038/nrneph.2010.156

10. de Boer IH, Katz R, Chonchol M, et al. Serum 25-hydroxyvitamin D and change in estimated glomerular filtration rate. *Clin J Am Soc Nephrol*. 2011;6(9):2141-2149. doi:10.2215/CJN.02640311

11. de Boer IH, Sachs MC, Cleary PA, et al; Diabetes Control and Complication Trial/Epidemiology of Diabetes Interventions and Complications Study Research Group. Circulating vitamin D metabolites and kidney disease in type 1 diabetes. *J Clin Endocrinol Metab*. 2012;97(12):4780-4788. doi:10.1210/jc.2012-2852

12. Miller ER III, Juraschek SP, Appel LJ, et al. The effect of n-3 long-chain polyunsaturated fatty acid supplementation on urine protein excretion and kidney function: meta-analysis of clinical trials. *Am J Clin Nutr*. 2009;89(6):1937-1945. doi:10.3945/ajcn.2008.26867

13. Hoogeveen EK, Geleijnse JM, Kromhout D, et al. Effect of omega-3 fatty acids on kidney function after myocardial infarction: the Alpha Omega Trial. *Clin J Am Soc Nephrol*. 2014;9(10):1676-1683. doi:10.2215/CJN.10441013

14. Elajami TK, Alfaddagh A, Lakshminarayan D, Soliman M, Chandnani M, Welty FK. Eicosapentaenoic and docosahexaenoic acids attenuate progression of albuminuria in patients with type 2 diabetes mellitus and coronary artery disease. *J Am Heart Assoc*. 2017;6(7):e004740. doi:10.1161/JAHA.116.004740

15. Momeni A, Mirhosseini M, Kabiri M, Kheiri S. Effect of vitamin D on proteinuria in type 2 diabetic patients. *J Nephropathol*. 2017;6(1):10-14. doi:10.15171/jnp.2017.03

16. Manson JE, Bassuk SS, Lee IM, et al. The Vitamin D and Omega-3 Trial (VITAL): rationale and design of a large randomized controlled trial of vitamin D and marine omega-3 fatty acid supplements for the primary prevention of cancer and cardiovascular disease. *Contemp Clin Trials*. 2012;33(1):159-171. doi:10.1016/j.cct.2011.09.009

17. Levey AS, Inker LA, Matsushita K, et al. GFR decline as an end point for clinical trials in CKD: a scientific workshop sponsored by the National Kidney Foundation and the US Food and Drug Administration. *Am J Kidney Dis*. 2014;64(6):821-835. doi:10.1053/j.ajkd.2014.07.030

18. Jun M, Turin TC, Woodward M, et al. Assessing the validity of surrogate outcomes for ESRD: a meta-analysis. *J Am Soc Nephrol*. 2015;26(9):2289-2302. doi:10.1681/ASN.2014040396

19. Inker LA, Schmid CH, Tighiouart H, et al; CKD-EPI Investigators. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367(1):20-29. doi:10.1056/NEJMoa1114248

20. Inker LA, Heerspink HJL, Tighiouart H, et al. GFR slope as a surrogate end point for kidney disease progression in clinical trials: a meta-analysis of treatment effects of randomized controlled trials. *J Am Soc Nephrol*. 2019;30(9):1735-1745. doi:10.1681/ASN.2019010007

21. Greene T, Ying J, Vonesh EF, et al. Performance of GFR slope as a surrogate end point for kidney disease progression in clinical trials: a statistical simulation. *J Am Soc Nephrol*. 2019;30(9):1756-1769. doi:10.1681/ASN.2019010009

22. Rubin DB. *Multiple Imputation for Nonresponse in Surveys*. New York, NY: John Wiley & Sons; 1987. doi:10.1002/9780470316696

23. Manson JE, Cook NR, Lee IM, et al; VITAL Research Group. Vitamin D supplements and prevention of cancer and cardiovascular disease. *N Engl J Med*. 2019;380(1):33-44. doi:10.1056/NEJMoa1809944

24. Manson JE, Cook NR, Lee IM, et al; VITAL Research Group. Marine n-3 fatty acids and prevention of cardiovascular disease and cancer. *N Engl J Med*. 2019;380(1):23-32. doi:10.1056/NEJMoa1811403

25. Coresh J, Turin TC, Matsushita K, et al. Decline in estimated glomerular filtration rate and subsequent risk of end-stage renal disease and mortality. *JAMA*. 2014;311(24):2518-2531. doi:10.1001/jama.2014.6634

26. de Borst MH, Hajhosseiny R, Tamez H, Wenger J, Thadhani R, Goldsmith DJ. Active vitamin D treatment for reduction of residual proteinuria: a systematic review. *J Am Soc Nephrol*. 2013;24(11):1863-1871. doi:10.1681/ASN.2013030203

27. Kim MJ, Frankel AH, Donaldson M, et al. Oral cholecalciferol decreases albuminuria and urinary TGF-β1 in patients with type 2 diabetic nephropathy on established renin-angiotensin-aldosterone system inhibition. *Kidney Int*. 2011;80(8):851-860. doi:10.1038/ki.2011.224

28. Ahmadi N, Mortazavi M, Iraj B, Askari G. Whether vitamin D_3 is effective in reducing proteinuria in type 2 diabetic patients? *J Res Med Sci*. 2013;18(5):374-377.