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Serum 25-hydroxy vitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes

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Abstract

Objective

People with diabetes frequently develop vascular disease. We investigated the relationship between blood 25-hydroxy vitamin D (25OH-D) concentration and vascular disease risk in type 2 diabetes.

Research design and methods

The relationships between blood 25OH-D concentration at baseline and the incidence of macrovascular (including myocardial infarction, stroke) and microvascular (retinopathy, nephropathy, neuropathy, and amputation) disease were analysed with Cox proportional-hazards models and logistic regression in an observational study of patients in the 5-year Fenofibrate Intervention and Event Lowering in Diabetes trial.

Results

50% of the patients had low vitamin D concentrations, as indicated by median blood 25OH-D concentration of 49nmol/L. These patients with a blood 25OH-D concentration < 50nmol/L had a higher cumulative incidence of macrovascular and microvascular events than those with levels ≥ 50nmol/L. Multivariate analysis, stratified by treatment and adjusted for relevant confounders, identified blood 25OH-D concentration as an independent predictor of macrovascular events. A 50nmol/L difference in blood 25OH-D concentration was associated with a 23% *(P*=0.007) change in risk of macrovascular complications during the study and further adjustments for seasonality, hs-CRP and physical activity level had little impact. The unadjusted risk of microvascular complications was 18% (*P*=0.006) higher during the study, though the excess risk declined to 11-14% and lost significance with adjustment for HbA1C, seasonality or physical activity.

Conclusions

Low blood 25OH-D concentrations are associated with an increased risk of macrovascular and microvascular disease events in type 2 diabetes. However, a causal link remains to be demonstrated.

Diabetes mellitus is among the leading causes of death and affects 347 million people worldwide. The WHO expects a 50% increase in deaths from diabetes over the next 10 years, and by 2030 diabetes mellitus is projected to be the seventh leading cause of death (1). Most major complications involve large vessel (macrovascular) or small vessel (microvascular) disease.

Recent reports have suggested associations between vascular disease (2–5), diabetes (6–8), and vitamin D deficiency. Higher rates of cardiovascular disease with lower vitamin D levels have also been reported, but most of these studies have been cross-sectional or based on unadjudicated endpoints. Rigorous large-scale prospective studies are needed to evaluate the relationship between circulating 25 hydroxyvitamin D (25OH-D) concentrations and vascular disease in diabetic patients, and more particularly, no large-scale studies have addressed the issue of microvascular diseases.

The FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study provides a unique opportunity to examine the relationship of blood vitamin D concentration with macrovascular and microvascular events (9–11). Here we report the relationships between baseline blood 25OH-D concentrations and prospective macrovascular and microvascular events in the FIELD cohort.

Research design and methods

Study design

The FIELD trial was a multinational, double-blind, placebo-controlled trial in 63 centres in Australia, New Zealand, and Finland. All patients gave written informed consent. The study protocol was approved by local and national ethics committees and undertaken in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

The study population (Supplemental figure S1) consisted of 9795 participants aged 50–75 years with type 2 diabetes mellitus diagnosed according to WHO criteria who were not taking lipid-modifying therapy at study entry. A detailed description of the study design has been published

(12). Patients recruited from hospital clinics and community sources were randomly allocated to once-daily micronised fenofibrate 200 mg (Laboratoires Fournier, Dijon, France) or matching placebo. Exclusion criteria included renal impairment (blood creatinine >130 μmol/L), known chronic liver disease or symptomatic gallbladder disease, and a cardiovascular event within the 3 months before recruitment. Blood 25OH-D results at baseline were available from 9524 FIELD participants (Supplemental figure 1).

Blood sampling and measurement of 25OH-D

Fasting blood samples were collected into serum tubes. After clotting, serum was separated and frozen on site. Frozen samples were transported to one of two central laboratories, in Adelaide (Australia) and Helsinki (Finland), where they were aliquoted and stored at -80°C until analysis. For the measurement of 25OH-D, frozen samples were transported to the Department of Biochemistry at Royal Prince Alfred Hospital in Sydney (Australia). Only 271 (2.8%) samples were unavailable for analysis. Blood 25OH-D was assayed by a chemiluminescence immunoassay (DiaSorin Spa, Italy) on a Liaison automated analyser (DiaSorin Spa, Saluggia, Italy). This assay detects 25OH-D_2 and 25OH-D_3 in an equimolar fashion. The lower limit of detection with this assay was 10 nmol/L; undetectable concentrations were assigned a value midway between this and zero (5 nmol/L). The assay used in this study was a premarket version that has been shown to compare closely with higher-order methods, including liquid chromatography tandem mass spectrometry (LCMS) and radioimmunoassay (13). In addition, it has been shown to have performance identical to that of the current market version of the DiaSorin Liaison assay. Assay performance was monitored over time by internal quality-control procedures including the use of high- and low-concentration pooled sera. Levels of 25OH-D below 65 nmol/L have been associated with rising levels of parathyroid hormone (14). While this may imply a broader agreement for the bone-related actions of 25OH-D, cut-off levels for many of the other actions of 25OH-D have not been established as yet. In agreement with previous

recommendations from the Institute of Medicine, 25 OH-D concentrations < 50 nmol/L were deemed to be low for the purposes of this report.

Assessment of other variables

Other biomarkers used for adjustment in analysis were measured using standardised routine methods as described previously (10). Measurement of high sensitive C-reactive protein (hs-CRP) used an automated immune-turbidometric assay from Roche Diagnostics run on a Modular E170 analyser (Roche Diagnostics, Mannheim, Germany). Baseline physical activity levels were recorded in response to a self-reported questionnaire including the categories self-care/home (e.g. house work, lawn mowing), occupational (type of work, e.g. sitting, home building), recreation (sports) and physical conditioning (aspects of general fitness). All categories were recorded on a scale from 1 (very light) to 5 (very heavy) (15).

Verification of macrovascular and microvascular disease events

A detailed description of all events and how they were verified has been published elsewhere (10,12). Macrovascular events included myocardial infarction, stroke, cardiovascular death, and coronary or carotid revascularisation. Incident events were verified by an outcome assessment committee masked to study treatment allocation. Incident microvascular events were classified as follows: peripheral neuropathy, abnormal monofilament test; nephropathy, urinary albumin:creatinine ratio ≥2.5mg/mmol for men and ≥3.5mg/mmol for women; retinopathy, on-study laser treatment for diabetic retinopathy (including macular oedema); amputation, minor amputation without known peripheral vascular disease in the same limb. Measurement of urinary albumin excretion and the monofilament test were performed at baseline, at 2 and 5 years, and at study close.

Statistical analysis

Normally distributed variables were summarised as means and standard deviations, and non-normally distributed variables as medians and interquartile ranges. Chi-square tests were used to

compare categorical variables. For continuous variables, groups were compared using *t* tests or Wilcoxon rank–sum tests if the variables were non-normally distributed. Spearman correlations were used for bivariate analysis of continuous variables.

Prospective associations with incident events during follow-up were assessed using unadjusted and adjusted Cox proportional-hazards analysis for macrovascular events, and logistic regression for microvascular events (as some of the latter were assessed only at specific time points). Analyses were stratified by study treatment allocation and adjusted for baseline event status. Adjusted models also included sex, age, diabetes duration, haemoglobin A1C, systolic blood pressure, body mass index (BMI), lipids (triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), smoking, baseline use of oral hypoglycaemics, and baseline use of insulin.

In exploratory analyses, we also adjusted for physical activity score, hs-CRP, and season. For each patient, date of sample collection and country of residence (northern or southern hemisphere) were used to identify the season at the time of sample collection (winter, spring, autumn, summer). Season was then included in the multivariable model as a factor variable.

As well as vitamin D in the models as a continuous variable (with results reported for increments of 50 nmol/L), quartiles of vitamin D were also examined. Cumulative risk curves of macrovascular and microvascular events were derived from the Kaplan–Meier method.

Two sided *P* values less than 0.05 were considered statistically significant. All analyses used SAS (version 9.2) and ACCorD (Analysis of Censored and Correlated Data) software (version 1.6.3).

Results

Baseline characteristics

The study treatment groups were well matched for baseline characteristics, such as age, body mass index, smoking habits, blood pressure and duration of diabetes (10). Both groups had

similar cardiovascular histories. The use of glucose-lowering and cardiovascular medication was as expected for a cohort of people with diabetes and did not differ between the two groups. The median 25OH-D concentration in the entire cohort was 49 nmol/L (range 5-196 nmol/L (Figure 1)) and there was no significant difference between the two groups. Eight percent, 52% and 89 % had blood 25OH-D concentrations <25, <50, and <75 nmol/L, respectively. Vitamin D supplements were used by 0.1% but only one patient used a dose >700 IU daily.

In patients who had low vitamin D at baseline (blood 25OH-D <50 nmol/L), cardiovascular disease, hypertension, retinopathy, and nephropathy were more prevalent (Table 1). In addition, these patients had a higher baseline prevalence of dyslipidaemia and microalbuminuria. On average, patients with low 25OH-D also had 0.3% (3 mmol/mol) higher HbA1c, 0.3 mg/L higher hs-CRP, and 1.2 kg/m^2 higher BMI. There were significantly fewer former and current smokers among the participants with low 25OH-D.

Description of events

During the 5-year follow-up period, 1250 patients (13.1% of the entire cohort) had at least one macrovascular event and 2395 (25.1% of the entire cohort) had at least one microvascular event. Most of the affected patients had one or two events during the 5-year follow-up. New or worsening nephropathy (1632 or 17.1% of the entire cohort) and new neuropathy (651or 6.8% of the entire cohort) were the most common microvascular complications. Retinopathy requiring laser surgery and amputations occurred in less than 5% of the entire cohort.

Prediction of incident macrovascular events by baseline blood 25OH-D

Blood 25OH-D was an independent predictor of incident macrovascular events over 5 years of follow-up. This relationship remained unchanged in multivariable analyses (Table 2). For each 50 nmol/L difference in baseline blood 25OH-D concentration, the unadjusted risk of incident cardiovascular events during follow-up was altered by 20% (95% CI, 5-39%; *P*=0.01). Multivariable adjustment confirmed a significant prospective association between baseline blood

25OH-D and incident cardiovascular events with a 23% (95% CI, 6-42%; *P*=0.007) higher risk per 50 nmol/L lower baseline concentration. Exploratory further adjustment for physical activity score, hs-CRP or seasonality level did not materially change these relationships (Table 3). The association between quartiles of blood 25OH-D and incident macrovascular events is shown in Table 2. In the adjusted model, patients in the lowest quartile of blood 25OH-D (<36 nmol/L) showed 21% higher risk of macrovascular disease than those in the highest quartile (>63 nmol/L).

Patients with a low blood 25OH-D concentration (less than the median (50 nmol/L)) had a higher cumulative incidence of macrovascular events than those whose concentrations fell above the median (Figure 2A). The cumulative incidence of microvascular disease was not significantly higher at the prespecified cut-point (Figure 2B), but a higher incidence was evident at levels of 25OH-D <36 nmol/L. Analysis using this cut-point, which was not pre-specified, revealed that the incidence was significantly higher in patients with 25OH-D levels below 36 nmol/L (Supplemental Figure 2).

Prediction of incident microvascular complications by baseline blood 25OH-D during follow-up

Blood 25OH-D was significantly associated with new microvascular complications over 5 years of follow-up (Table 2). Compared with those whose blood 25OH-D concentrations fell above the median (50 nmol/L), the lower group had an absolute excess of events during follow-up (Figure 2). Prospective analysis of incident events during follow-up revealed 18% (95% CI, 5-32%; *P*=0.006) higher unadjusted risk of a microvascular event for each 50 nmol/L difference in 25OH-D blood concentration, and a 15% (95% CI, 1-30%; *P*=0.03) higher risk after multivariable adjustment. Exploratory further adjustment for HbA1c, physical activity score, or seasonality abolished its significance and reduced the incremental risk to 11-12% (not significant, Table 3).

Discussion

This large-scale prospective study demonstrates an inverse association between the serum concentration of 25OH-D and future macrovascular and microvascular disease which was independent of treatment and the duration of diabetes. Higher baseline blood 25OH-D was associated with reductions in the risk of macrovascular disease by 20 % and microvascular disease by 18% per 50 nmol/L, which, after adjustment for classical risk predictors, including HbAIC, became 23 % and 11% (not significant), respectively. 25OH-D levels below 36 nmol/L were significantly associated with future microvascular events, but analysis of this cutpoint was based on visualization of the distribution of events and was not prespecified. Whilst it raised the possibility that more severe 25OH-D depletion may contribute directly to the risk of microvascular events, analysis adjusted for HbA1c still fell short of significance (P = 0.07).

The results are in keeping with cross-sectional (5) and prospective (4) studies showing an inverse relationship between 25OH-D and cardiovascular disease. A cross-sectional analysis of data from the third National Health and Nutrition Examination Survey (NHANES) showed a higher prevalence of vitamin D deficiency in patients with cardiovascular disease than in patients without (29.3 vs 21.4 %) (5). In addition, subjects with low 25OH-D had an odds ratio for pre-existing cardiovascular disease of 1.20 (95 % CI, 1.01-1.36; P=0.03). However, the study was cross-sectional and lacked a rigorous verification of cardiovascular disease. In a nested case-control study including 454 patients with cardiovascular events and 900 matched controls, individuals with vitamin D deficiency at baseline had a 10-year relative risk of nonfatal myocardial infarction or fatal coronary heart disease of 2.09 (95 % CI, 1.24-3.54; P=0.02 for trend) (4). Case-control studies typically overestimate risk, are affected by selection bias and lack randomisation. In contrast, FIELD was a prospective, randomised study with 1250 cardiovascular events rigorously verified by an independent event review committee.

The results of our study expand existing knowledge by showing prospectively in a large cohort that low blood 25OH-D is also a risk factor for development of peripheral neuropathy, retinopathy, nephropathy, and amputation. Previous cross-sectional studies reported lower circulating 25OH-D concentrations in diabetic patients with microvascular disease events (16–19). However, all these studies were limited by their size and design. In NHANES, for example, the diagnosis of peripheral neuropathy is based on self-reported symptoms of neuropathy, such as pain, tingling, numbness, or loss of feeling in hands or feet (19). In contrast, Kaur et al. reported a higher prevalence of vitamin D deficiency in patients with diabetic retinopathy but found no association between vitamin D deficiency and neuropathy or nephropathy (20).

Potential mechanisms that explain the relationship between vitamin D deficiency and vascular disease (microvascular and macrovascular disease) include pancreatic beta-cell dysfunction, peripheral insulin resistance, chronic inflammation and endothelial dysfunction. In animal models, vitamin D deficiency impairs insulin synthesis (21, 22), possibly via a reduced intracellular calcium concentration (23). The final statistical models presented in our study were adjusted for indices of glycaemic control, such as glycated haemoglobin, fasting glucose and insulin, so beta-cell dysfunction and abnormal glucose homoeostasis may partly explain the increased risk of microvascular, but not macrovascular disease, with decreasing blood concentrations of 25OH-D.

Despite the well-established link between immune function and vitamin D (24), reduction in blood 25OH-D concentrations may be a nonspecific marker of chronic illness and the associations between blood 25OH-D and clinical outcomes may represent reverse causality. In our study blood 25OH-D remained a significant predictor of macrovascular and microvascular events after adjustment for hs-CRP, a biomarker of chronic inflammation. Recent metanalyses failed to find benefits for survival, cardiovascular disease or multiple outcomes (other than birthweight) following vitamin D supplementation (25, 26). On the other hand, metanalyses

which differentiated between D2 and D3 supplementation demonstrated a selective benefit following the use of D3 (27, 28). In the present study, blood 25OH-D measurements reflected the concentration of the combination of D2 and D3.

Sedentary lifestyle characterised by insufficient physical activity is another established risk factor for cardiovascular disease and type 2 diabetes. Physical activity may increase blood 25OH-D concentrations as a consequence of an associated increase in sunlight exposure. Sarcopoenia and reduced muscle strength are associated with low blood 25OH-D concentrations (29) and a number of recent publications support the presence of the vitamin D receptor (VDR) in numerous tissues including skeletal muscle (30, 31). The study by Autier detected a beneficial effect of lower doses of vitamin D among elderly women in whom mobility was more likely to be reduced (25). Adjustment for activity level affected the inverse association between vitamin D and microvascular, but not macrovascular, outcomes.

Direct effects of vitamin D on vessel function are another explanation for the relationship between vitamin D and vascular disease (33-34). However, Wang and DeLuca recently reported that the VDR is not expressed in cardiac myocytes and vascular smooth muscle cells (35), which challenges the hypothesis of direct vascular effects of vitamin D. In fact, it appears that most of the antibodies used to identify VDR in these previous studies detect proteins other than VDR, and may have led to false-positive results. In summary, existing data are insufficient to establish the mechanism responsible for the increased risk of vascular events in people with low vitamin D. However, the present results suggest that the relationship between serum 25OH-D and microvascular complications is partially due to the surrogacy for the effects of diabetes, season and activity.

The median blood 25OH-D concentration in the FIELD cohort was 49 nmol/L, indicating that more than 50 % of the participants patients had low 25OH-D . Furthermore, 25% had blood

25OH-D concentrations of 36 nmol/L or less. This supports previous reports showing a high prevalence of vitamin D deficiency in the general population and in elderly individuals worldwide (36–38). Considering the relationship of vitamin D with common chronic diseases, the high prevalence of vitamin D deficiency has socioeconomic implications, affecting individual patients and health care systems.

The main strengths of the study are its prospective and randomised design, the large number of participants and the high number of verified microvascular and macrovascular events. Blood 25OH-D was measured with a well characterised latest generation chemiluminescence immunoassay that compares well with higher-order methods, such as liquid chromatography tandem mass spectrometry (13). This is important, as the performance of vitamin D assays varies substantially (13,39).

Our study also has some limitations. Fenofibrate treatment is known to reduce cardiovascular and microvascular events and may have biased our results. However, the effect of blood 25OH-D on microvascular and macrovascular risk was similar in the placebo and the fenofibrate groups, and no heterogeneity was detected. Individuals with low 25OH-D had a higher prevalence of microvascular and macrovascular disease at baseline which predisposes them to a higher event rate during the study period, but there was no difference in the association seen among people with and without prior macrovascular and microvascular disease.

These relationships raise the question whether vitamin D supplementation can lower the risk of vascular events (25, 26). Few prospective intervention studies with sufficient statistical power have addressed this question. Both the Womens Health Initiative Study, which followed over 36 000 women, and the RECORD trial, which studied over 5000 patients, failed to show cardiovascular benefit (40, 41), but metanalysis suggests the formulation of vitamin D may be important (27, 28).

The findings also raise the question whether vitamin D supplementation can lower the risk of diabetic microvascular complications, possibly via an effect on $HbA1_C$. Short-term administration of a supraphysiologic dose of vitamin D does not affect insulin sensitivity (42). In contrast, supplementation with 2000 IU of cholecalciferol for 16 weeks has been reported to improve ß-cell function (43). A recent meta-analysis suggested that vitamin D administration did not improve HbA1c or overall mortality (25). Together with our results, the negative outcomes of vitamin D supplementation studies highlight the importance of endogenous vitamin D synthesis and the need for further research into the role of vitamin D deficiency.

In conclusion, this study provides prospective evidence for serum vitamin D as a risk marker for future vascular events. The association of vitamin D with macrovascular disease was not explained by associated effects on β-cell function, glucose homeostasis, physical activity or seasonality whilst the novel relationship between serum vitamin D and microvascular disease was attributable to these covariates. The mechanisms underlying these relationships require further research and the results of large-scale supplementation trials.

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Authors' contributions

MH, DRS and ACK conceived the study. MH and DRS wrote the paper. ASV contributed to the design of the study and analysed the data. TMcC, IRS, RS, ML, DT, AJJ, SB, and MW interpreted the data and revised the paper. TMcC and IRS established and performed the laboratory analyses.

Conflicts of interest

None of the authors has any conflicts of interest to declare with respect to this study.

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Table 1: Baseline characteristics by serum vitamin D concentration (≥50 vs <50)

Characteristic	Total vitamin D ≥50 nmol/L (*n*=4560)	Total vitamin D <50 nmol/L (*n*=4964)
General characteristics		
Assigned fenofibrate	2266 (49.7%)	2495 (50.3%)
Male	3235 (70.9%)	2732 (55.0%)*
Age at visit 1 (years, mean[SD])	62.5 (6.8)	62.0 (7.0)*
Diabetes duration (years, median[IQR])	5 (2–9)	5 (2–10)*
Body-mass index (kg/m2, median[IQR])	29.2 (26.5–32.6)	30.4 (27.1–34.5)*
Blood pressure, systolic (mmHg, mean[SD])	139.8 (15.0)	141.2 (15.7)*
Blood pressure, diastolic (mmHg, mean[SD])	81.7 (8.3)	82.4 (8.7)*
Exsmoker or current smoker	2849 (62.5%)	2860 (57.6%)*
Clinical history		
Previous cardiovascular disease	947 (20.8%)	1120 (22.6%)*
Peripheral vascular disease	341 (7.5%)	400 (8.1%)
Coronary revascularisation (CABG or PTCA)	195 (4.3%)	154 (3.1%)*
History of hypertension	2498 (54.8%)	2907 (58.6%)*
History of diabetic retinopathy	345 (7.6%)	448 (9.0%)*
Neuropathy (self-reported)	574 (12.6%)	789 (15.9%)*
Laboratory data		
Total cholesterol (mmol/L, mean[SD])	4.98 (0.71)	5.09 (0.70)*
LDL cholesterol (mmol/L, mean[SD])	3.05 (0.66)	3.07 (0.64)
HDL cholesterol (mmol/L, mean[SD])	1.08 (0.25)	1.11 (0.27)*
Triglycerides (mmol/L, median[IQR])	1.68 (1.31–2.24)	1.79 (1.38–2.40)*
HbA1c (%,median[IQR])	6.7 (6.0–7.6)	7.0 (6.2–8.0)*
HbA1c (mmol/mol)	50 (42–60)	53 (44–64)
Plasma creatinine (umol/L, mean[SD])	79.1 (15.5)	76.3 (15.9)*
Dyslipidaemia	1624 (35.6%)	1982 (39.9%)*
Urine ACR, microalbuminuria	921 (20.3%)	1128 (22.8%)*
Urine ACR, macroalbuminuria	185 (4.1%)	210 (4.2%)
Hight sensitive C-reactive protein (mg/L, median[IQR])	2.6 (1.3–5.5)	2.9 (1.3–6.3)*
Baseline cardiovascular medication		
Any antithrombotic	1432 (31.4%)	1547 (31.2%)
Any warfarin	119 (2.6%)	114 (2.3%)
Aspirin or other antithrombotic without warfarin	1313 (28.8%)	1433 (28.9%)

Characteristic	Total vitamin D ≥50 nmol/L (*n*=4560)	Total vitamin D <50 nmol/L (*n*=4964)
Angiotensin II receptor antagonist	241 (5.3%)	273 (5.5%)
ACE inhibitor	1460 (32.0%)	1741 (35.1%)*
Beta blocker	602 (13.2%)	784 (15.8%)*
Calcium antagonist	878 (19.3%)	956 (19.3%)
Diuretic	658 (14.4%)	795 (16.0%)*
Nitrate	230 (5.0%)	303 (6.1%)*
Baseline blood-glucose-lowering medication		
Diet	1309 (28.7%)	1220 (24.6%)*
Oral hypoglycaemic agent	2724 (59.7%)	2955 (59.5%)
Any insulin	527 (11.6%)	789 (15.9%)*
Vitamin D supplementation		
Vitamin D supplements	3 (0.1%)	4 (0.1%)
Vitamin D <700 IU daily	3 (0.1%)	3 (0.1%)
Vitamin D ≥700 IU daily	0 (0.0%)	1 (0.0%)
Calcium supplements	123 (2.7%)	137 (2.8%)

* $P<0.05$

ACR, albumin/creatinine ratio

Table 2: Association between serum vitamin D concentration (25OH-D) and new events during follow-up

				Unadjusted model			Adjusted model		
	n	Events	%	OR or HR (95% CI)	*P*	*P* for trend	OR or HR (95% CI)	*P*	*P* for trend
Macrovascular									
25OH-D (nmol/L) (per 50 nmol/L difference)	9524	1250	13.12	1.20 (1.05–1.39)†	0.01		1.23 (1.06–1.42) †	0.007	
Quartile						0.02			0.01
>63	2354	293	12.45	1.00†			1.00†		
50–63	2206	269	12.19	0.99 (0.84–1.17)†	0.89		1.00 (0.84–1.18) †	0.97	
36–<50	2680	353	13.17	1.07 (0.91–1.24)†	0.42		1.10 (0.94–1.28) †	0.25	
<36	2284	335	14.67	1.19 (1.02–1.40)†	0.03		1.21 (1.03–1.43) †	0.02	
Microvascular									
25OH-D (nmol/L) (per 50 nmol/L difference)	9524	2395	25.15	1.18 (1.05–1.32)†	0.006		1.11 (0.98–1.26) †	0.11	
Quartile						0.008			0.13
>63	2354	568	24.13	1.00†			1.00†		
50–63	2206	526	23.84	0.98 (0.86–1.13)†	0.82		0.95 (0.82–1.09) †	0.46	
36–<50	2680	679	25.34	1.07 (0.94–1.21)†	0.32		1.02 (0.89–1.16) †	0.81	
<36	2284	622	27.23	1.18 (1.03–1.34)†	0.02		1.10 (0.95–1.26) †	0.19	

* Odds ratio, adjusted for age, sex, diabetes duration, haemoglobin A1c, systolic blood pressure, body mass index, lipids (triglycerides, high- and low-density lipoprotein cholesterol), smoking, baseline use of oral hypoglycaemics, and baseline use of insulin.

† Hazard ratio, stratified by treatment and adjusted for prior macrovascular disease, age, sex, diabetes duration, haemoglobin A1c, systolic blood pressure, body mass index, lipids (triglycerides, high- and low-density lipoprotein cholesterol), smoking, baseline use of oral hypoglycaemic agents, and baseline use of insulin.

Table 3: Exploratory models investigating the impact of HbA1c, hs-CRP, physical activity and seasonal variability on the association between serum 25OH-D and new events during follow-up

		Adjusted model	
New events during follow-up		**OR or HR (95% CI)**	***P***
Macrovascular			
25OH-D (nmol/L) (per 50 nmol/L difference)	Adjusted for all* + HbA1c	1.23 (1.06–1.42)	0.007
	Adjusted for all* + hs-CRP	1.26 (1.09-1.46)	0.002
	Adjusted for all* + physical activity	1.21 (1.04-1.42)	0.02
	Adjusted for all* + seasonal variability	1.24 (1.06-1.44)	0.006
Microvascular			
25OH-D (nmol/L) (per 50 nmol/L difference)	Adjusted for all* + HbA1c	1.11 (0.98–1.26)	0.11
	Adjusted for all* + hs-CRP	1.14 (1.01-1.29)	0.04
	Adjusted for all* + physical activity	1.11 (0.98-1.27)	0.11
	Adjusted for all* + seasonal variability	1.12 (0.99-1.27)	0.08

* Stratified by treatment and adjusted for age, sex, diabetes duration, systolic blood pressure, body mass index, lipids (triglycerides, high- and low-density lipoprotein cholesterol), smoking, baseline use of oral hypoglycaemics, and baseline use of insulin.

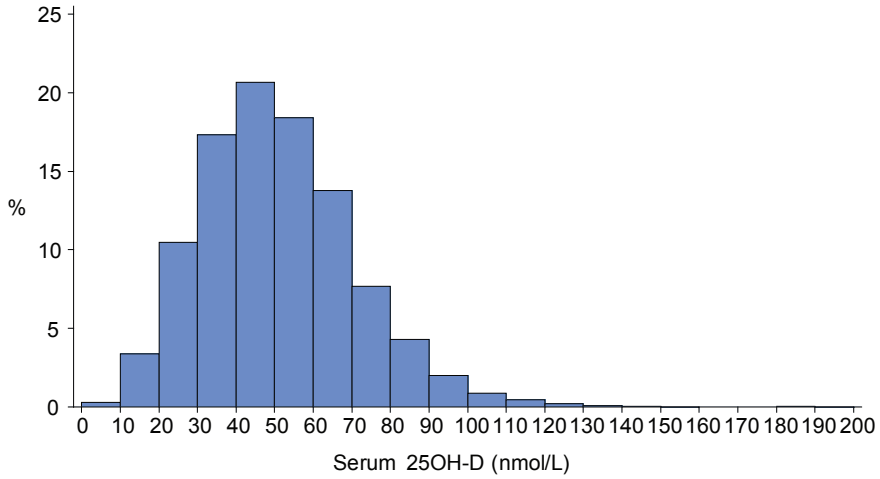
Figure legends

Figure 1

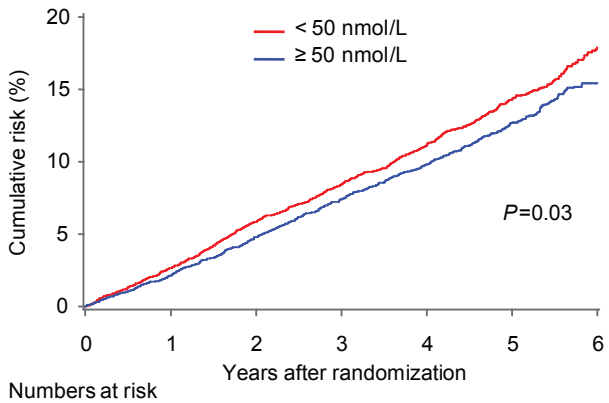
Distribution of baseline blood 25-hydroxy vitamin D (25OH-D) concentrations in the FIELD study.

Figure 2

Effects of serum concentration of 25-hydroxy vitamin D (25OH-D) on incident events. A. Cumulative risk of macrovascular events for patients above and below the median 25OH-D concentration. B. Cumulative risk of microvascular events for patients above and below the median 25OH-D concentration. Complete microvascular event data were available at 2 years, 5 years, and at end-of-study follow-up.


25
20
15
%
10
5
0
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200
Serum 25OH-D (nmol/L)

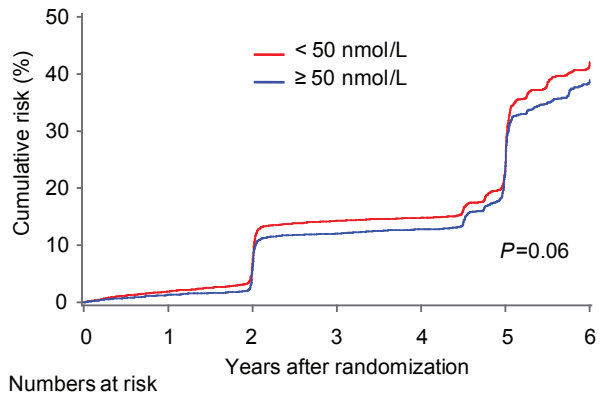
A



Numbers at risk

—	4964	4817	4638	4481	4321	2430	779
—	4560	4453	4300	4166	4016	2097	734

B



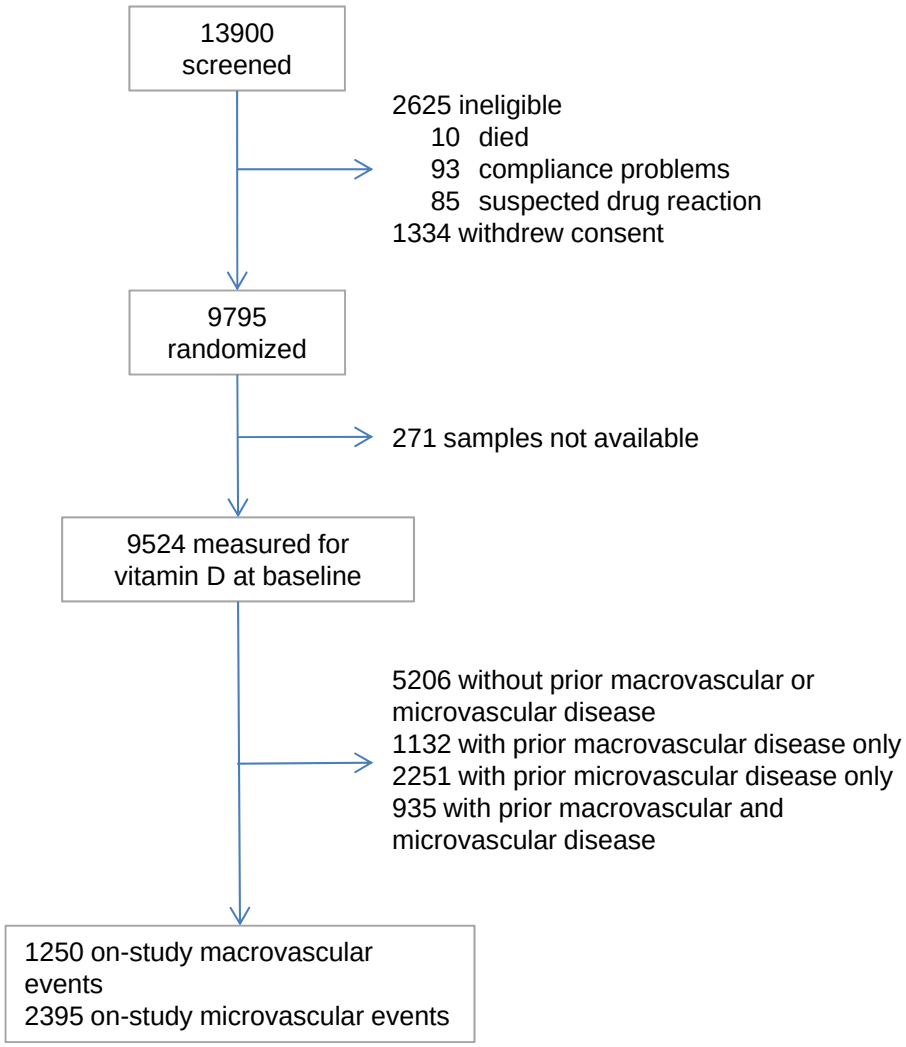
Numbers at risk

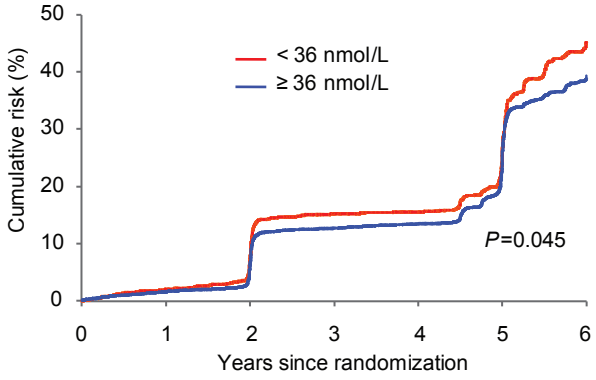
—	4964	4831	4532	4160	4079	2264	691
—	4560	4478	4227	3941	3848	1923	640

Serum 25-hydroxy vitamin D: a predictor of macrovascular and microvascular complications in patients with type 2 diabetes

Supplemental figure S1

Patient enrolment and progress through the study.



Supplemental figure S2

Effects of serum concentration of 25OH-D <36 nmol/L or ≥36 nmol/L on incident microvascular disease events (complete microvascular event data were available at 2 years, 5 years, and at end-of-study follow-up).