Effect of active vitamin D treatment on development of type 2 diabetes: DPVD randomised controlled trial in Japanese population

Tetsuya Kawahara,[1,2] Gen Suzuki,[3] Shoichi Mizuno,[4] Tetsuya Inazu,[5] Fumiyoshi Kasagi,[6] Chie Kawahara,[1] Yosuke Okada,[1] Yoshiya Tanaka[1]

[1]University of Occupational and Environmental Health, Kitakyushu, Japan
[2]Shin Komonji Hospital, Kitakyushu, Japan
[3]International University Health and Welfare Clinic, Ohtawara, Japan
[4]National Cancer Center EPOC, Kashiwa, Japan
[5]Ritsumeikan University, Kusatsu, Japan
[6]Radiation Effects Association, Tokyo, Japan

Correspondence to: T Kawahara
k-tetsuy@med.uoeh-u.ac.jp
(ORCID 0000-0002-1268-4871)

Additional material is published online only. To view please visit the journal online.

Cite this as: *BMJ* 2022;377:e066222
http://dx.doi.org/10.1136/bmj-2021-066222

Accepted: 07 April 2022

ABSTRACT

OBJECTIVE

To assess whether eldecalcitol, an active vitamin D analogue[2], can reduce the development of type 2 diabetes among adults with impaired glucose tolerance.

DESIGN

Double blinded, multicentre, randomised, placebo controlled trial.

SETTING

Three hospitals in Japan, between June 2013 and August 2019.

PARTICIPANTS

People aged 30 years and older who had impaired glucose tolerance defined by using a 75 g oral glucose tolerance test and glycated haemoglobin level.

INTERVENTIONS

Participants were randomised to receive active vitamin D (eldecalcitol 0.75 µg per day; n=630) or matching placebo (n=626) for three years.

MAIN OUTCOMES

The primary endpoint was incidence of diabetes. Prespecified secondary endpoints were regression to normoglycaemia and incidence of type 2 diabetes after adjustment for confounding factors at baseline. In addition, bone densities and bone and glucose metabolism markers were assessed.

RESULTS

Of the 1256 participants, 571 (45.5%) were women and 742 (59.1%) had a family history of type 2 diabetes. The mean age of participants was 61.3 years. The mean serum 25-hydroxyvitamin D concentration at baseline was 20.9 ng/mL (52.2 nmol/L); 548 (43.6%) participants had concentrations below 20 ng/mL (50 nmol/L). During a median follow-up of 2.9 years, 79 (12.5%) of 630 participants in the eldecalcitol group and 89 (14.2%) of 626 in the placebo group developed type 2 diabetes (hazard ratio 0.87, 95% confidence interval 0.67 to 1.17; P=0.39). Regression to normoglycaemia was achieved in 145 (23.0%) of 630 participants in the eldecalcitol group and 126 (20.1%) of 626 in the placebo group (hazard ratio 1.15, 0.93 to 1.41; P=0.21). After adjustment for confounding factors by multivariable fractional polynomial Cox regression analysis, eldecalcitol significantly lowered the development of diabetes (hazard ratio 0.69, 0.51 to 0.95; P=0.020). In addition, eldecalcitol showed its beneficial effect among the participants with the lower level of basal insulin secretion (hazard ratio 0.41, 0.23 to 0.71; P=0.001). During follow-up, bone mineral densities of the lumbar spine and femoral neck and serum osteocalcin concentrations significantly increased with eldecalcitol compared with placebo (all P<0.001). No significant difference in serious adverse events was observed.

CONCLUSIONS

Although treatment with eldecalcitol did not significantly reduce the incidence of diabetes among people with pre-diabetes, the results suggested the potential for a beneficial effect of eldecalcitol on people with insufficient insulin secretion.

TRIAL REGISTRATION

UMIN Clinical Trials Registry UMIN000010758.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Observational studies have shown that vitamin D deficiency is associated with insulin resistance and an increased risk of future diabetes

However, results of randomised controlled trials of vitamin D supplementation for preventing type 2 diabetes are not consistent

A previous study suggested that vitamin D supplementation was beneficial for people with pre-diabetes and vitamin D deficiency

WHAT THIS STUDY ADDS

Active vitamin D might be beneficial for people with insufficient insulin secretion among the pre-diabetic population

Treatment with eldecalcitol was effective in increasing bone mineral densities and serum osteocalcin concentrations

Introduction

Diabetes mellitus is a major risk factor for various cardiovascular and renal diseases. The global prevalence of diabetes was 425 million adults in 2015, with an anticipated increase to 629 million by 2040.[1] Moreover, a further 352 million people with impaired glucose tolerance are at high risk for developing diabetes and cardiovascular disease.[1 2] Although lifestyle modifications may delay the development of type 2 diabetes,[3 4] maintaining long term behavioural changes is difficult.[5 6] Therefore, new strategies to reduce the incidence of type 2 diabetes are needed for people with impaired glucose tolerance.

Vitamin D receptors have been found in various cell types, including the pancreatic β cells,[7 8] and active vitamin D is reportedly involved in insulin biosynthesis and secretion.[9] Genetic studies in mice have shown that inter-organ communication and bone metabolism seem to be closely associated with insulin resistance.[10-12] Observational studies

have shown an association between low serum 25-hydroxyvitamin D concentrations and increased incidence of type 2 diabetes.[13 14] Although several intervention studies and a recent meta-analysis have suggested that vitamin D supplementation may have a beneficial effect on glycaemic control,[15-18] relatively large scale clinical studies and other meta-analyses of randomised clinical trials have not supported this finding.[19-23] Whether vitamin D supplementation is beneficial only for children with vitamin D deficiency is unclear,[20] as is which bone metabolism markers may be associated with the improvement in insulin resistance after treatment. Hence, we did a prospective trial, the Diabetes Prevention with active Vitamin D (DPVD) study, to assess whether 0.75 µg per day of eldecalcitol, an active vitamin D analogue, could reduce the incidence of type 2 diabetes among people with impaired glucose tolerance.

Methods

This randomised, double blind, placebo controlled trial evaluated the effect of eldecalcitol on incidence of type 2 diabetes in people with impaired glucose tolerance in Japan. It was designed and supervised by the steering committee and approved by the institutional review boards at three participating centres. The trial protocol has been previously published.[24] Written informed consent was obtained from all participants before enrolment in the trial.

Study participants

Male and female patients who were aged 30 years or older and had impaired glucose tolerance were recruited to the study. We defined impaired glucose tolerance as meeting all three of the following glycaemic criteria: fasting glucose concentration <126 mg/dL (7.0 mmol/L), two hour glucose concentration 140-199 mg/dL (7.8-11.0 mmol/L) during a 75 g oral glucose tolerance test, and glycated haemoglobin <6.5% (48 mmol/L).[25 26] The complete list of inclusion and exclusion criteria, published previously,[24] is provided in the supplementary appendix.

Randomisation and masking

Sub-investigators in the three trial hospitals applied to the assignment centre for registration and treatment assignment. Participants were assigned to one of two treatment groups in a one to one ratio by using a central randomisation method. A responsible person at the assignment centre made a randomisation list for each hospital separately by using a stratified permuted block procedure before the first participant's entry. The number of strata was eight according to sex (male, female), age (30-54 years, ≥55 years), and 75 g oral glucose tolerance test two hour post-load plasma glucose (<170 mg/dL, ≥170 mg/dL), because we considered these factors to affect the incidence of diabetes. The block sizes were six for five permute cycles and four for the next five permute cycles, and the last 17 participants were allocated using two block sizes—that is, a total of 417 participants per hospital. On the basis of the assignment list, which was kept in a locked safe located in the assignment centre, the responsible person enrolled and allocated participants to either the eldecalcitol group or the placebo group in the order of registration. The assignment list was inaccessible to the investigators or sub-investigators for the duration of the trial except in the event of emergencies. The key was retrieved only after the trial concluded and data were fixed. Participants were randomly assigned to take a single, once daily, hard gel pill containing either 75 µg of eldecalcitol or matching placebo, which looks exactly the same. A standard dose of 0.75 µg of eldecalcitol is used for the prevention and/or treatment of osteoporosis in Japan. Eldecalcitol and placebo were prescribed by a sub-investigator (physician) at every three monthly visit and exchanged for the prescription at an independent pharmacy that had no association with any members of the DPVD Research Group. The placebo was purchased from Sunsho Pharmaceutical, which was responsible for the manufacturing, packing, and distribution of the placebo. This company had no role in the design or conduct of the trial.

Procedures

Study visits were scheduled at three month intervals, with the follow-up period concluding after three years. A routine clinical examination, including measurement of fasting plasma glucose and glycated haemoglobin, was performed at each study visit. Each participant received a brief (five to 10 minutes long) talk on appropriate calorie intake from diet and exercise at each study visit, using an information sheet. A 75 g oral glucose tolerance test, serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations, and bone mineral density were measured at baseline and at yearly intervals. Serum 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D concentrations were measured by liquid chromatography-tandem mass spectrometry at LSI Medience Corporation (Tokyo, Japan). All data were collected in the assignment centre.

Outcomes

The primary endpoint was the development of type 2 diabetes, defined as meeting at least two of the following criteria: glycated haemoglobin ≥6.5%, fasting plasma glucose concentration ≥126 mg/dL, two hour post-load plasma glucose concentration ≥200 mg/dL (11.1 mmol/L), or random plasma glucose concentration ≥200 mg/dL. The first secondary endpoint was the regression to normoglycaemia, defined as meeting all three glycaemic criteria—glycated haemoglobin <6.5%, fasting plasma glucose concentration <110 mg/dL (6.1 mmol/L), and two hour post-load plasma glucose concentration <140 mg/dL—or both of the following criteria: glycated haemoglobin <5.7% and fasting plasma glucose concentration <100 mg/dL.[27] The other secondary endpoint was the hazard ratio of eldecalcitol compared with placebo for the incidence of type 2 diabetes after adjustment for 11 confounding factors at baseline: age, sex (male/female), presence or

doi: 10.1136/bmj-2021-066222 | BMJ 2022;377:e066222 | the bmj

absence of hypertension (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or both), body mass index, family history of diabetes (yes/no), glycated haemoglobin, fasting plasma glucose, two hour plasma glucose, 25-hydroxyvitamin D, homoeostasis model assessment of insulin resistance (HOMA-IR), and insulinogenic index.

Statistical analysis

We assessed the intention-to-treat population, comprising all participants who were randomised and received at least one dose of the study drug, for the primary analysis. However, we also show the results of Cox regression analyses using the per protocol population and the "complete" population in the sensitivity analyses. The complete population was a study population with a complete set of measurements throughout the three year study period, which excluded participants who dropped out other than those who achieved the normal glucose tolerance status. We analysed progression to diabetes and regression to normoglycaemia with the log-rank test and two by four χ^2 test, respectively. We compared treatments by estimation of hazard ratios and 95% confidence intervals. Additionally, we did Cox regression analysis using a multivariable fractional polynomial (mfp) method by an mfp package for R with eldecalcitol and the 11 baseline covariates, described above.[28] We did subgroup analyses of continuous covariables by using splines with an mgcv package for R.[29]

Values were missing for covariates such as body weight, blood pressure, glycated haemoglobin, plasma glucose concentrations. To evaluate the time trends of covariates, we used a multiple imputation method for replacing missing values separately in each group with other plausible values by creating multiple filling-in patterns to avoid bias caused by missing data.[30] In this study, we replaced each missing value with a set substituted plausible value by 20 filled-in complete datasets by using a multiple imputation by chained equation method.[31] In the imputation process, we used the following covariates to create 20 complete datasets: age, sex, body mass index, blood pressure, family history of diabetes, glycated haemoglobin, plasma glucose, HOMA-IR, lipids concentrations, serum 25-hydroxyvitamin D concentrations, and serum 1,25-dihydroxyvitamin D concentrations in each group. We used Rubin's rules to calculate standard errors.[32] These standard errors take into account the variability in results between imputed datasets and reflect the uncertainty associated with the missing data.[33] We averaged estimated associations in each of the imputed datasets together to give overall estimated associations. We also did a repeated measure analysis of variance and Dunnett test for the time trends of measurements.

In the original trial design, approximately 750 patients were needed on the basis of the following assumptions: 8.4% per year incidence of diabetes in the placebo group, participant accrual period of 2.3 years, study duration of 5.3 years, and a 7% dropout rate. The study had 80% power to detect a 36% lower rate of the primary endpoint in the active vitamin D group than in the placebo group, with a two sided type I error

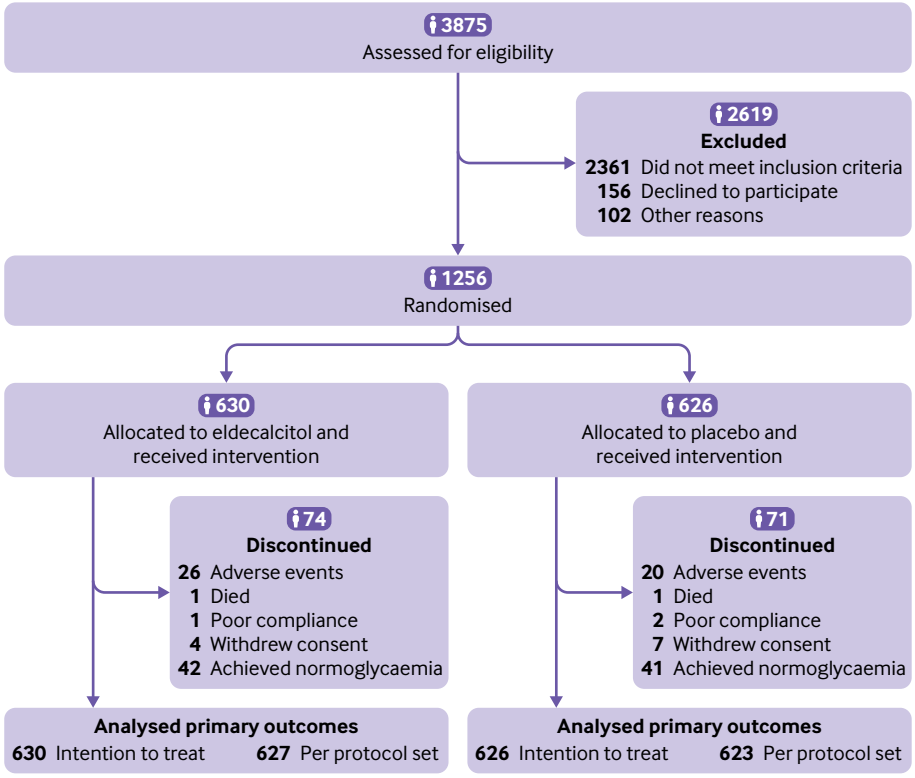

Fig 1 | Flow diagram of study

of 0.05. However, in the middle of the study, regression to normoglycaemia occurred, and the dropout ratio of participants who reached the normoglycaemic state was greater than anticipated, suggesting that the number of participants developing diabetes would be much smaller than originally planned. Therefore, we amended the protocol so that the cumulative incidence of type 2 diabetes in the control group was 16.9% (6.0% annually)—that is, the active vitamin D group was 11.1% (3.8% annually) and the dropout ratio was 12% in the recruitment phase of the study. Thus, the relative risk reduction was assumed at 36%. As a result, 625 participants were needed in each group (a total of 1250).

We used R software, version 4.05, for statistical analyses. We considered two sided P values less than 0.05 to be statistically significant.

Patient and public involvement

Patients and the public were not involved in setting the research question or outcome measures or in the writing of the results. However, patient representatives asked us to measure bone mineral densities for all participants yearly. As a result, we added the annual measurement of bone mineral density to the protocol.

Results

From 1 June 2013 through 31 August 2015, a total of 3875 participants were recruited from 32 institutions in Japan (fig 1), and 1256 participants were randomly assigned to receive either eldecalcitol (630 participants) or placebo (626 participants) (621 participants had impaired glucose tolerance alone and 635 had impaired glucose tolerance with impaired fasting glucose). Of the 1256 participants, 45.5% were women and 59.1% had a family history of type 2 diabetes. The mean age of participants was 61.3 (range 30-78) years. No clinically relevant differences in baseline characteristics existed between the two groups (table 1; supplementary table A). The mean serum 25-hydroxyvitamin D concentration at baseline was 20.9 ng/mL (52.2 nmol/L); 43.6% of participants had concentrations of <20 ng/mL (50 nmol/L). The classification of vitamin D concentrations in Japan is ≥30 ng/mL (≥75 nmol/L) as normal, 20 to <30 ng/mL (50 to <75 nmol/L) as insufficiency, and <20 ng/mL (<50 nmol/L) as deficiency.[34-36] The trial was finished in August 2019. The median follow-up was 2.9 (interquartile range 2.8-3.0) years.

Table 1 | Baseline characteristics of study participants. Values are means (standard deviations) unless stated otherwise

Characteristic	Overall (n=1256)	Eldecalcitol (n=630)	Placebo (n=626)
Age, years	61.3 (8.9)	61.1 (8.8)	61.4 (9.1)
No (%) female sex	571 (44.5)	288 (45.7)	283 (45.2)
Body mass index	24.3 (2.3)	24.1 (2.7)	24.5 (1.8)
Blood pressure, mm Hg:			
Systolic	134.3 (11.0)	134.4 (12.4)	134.2 (9.4)
Diastolic	82.9 (9.1)	82.8 (10.3)	83.1 (7.6)
No (%) family history of diabetes	742 (59.1)	380 (60.3)	362 (57.8)
Glycated haemoglobin, %	6.0 (0.2)	5.9 (0.2)	6.0 (0.2)
Plasma glucose, mg/dL:			
Fasting state	109.9 (9.2)	110.0 (9.5)	109.8 (8.9)
30 min after oral glucose load	176.3 (20.1)	177.4 (19.8)	175.3 (20.2)
2 h after oral glucose load	168.4 (17.8)	168.9 (20.1)	168.0 (15.0)
Plasma insulin, μU/dL:			
Fasting state	6.9 (2.6)	7.0 (3.0)	6.8 (2.2)
30 min after oral glucose load	49.4 (23.2)	48.5 (24.9)	50.3 (21.3)
2 h after oral glucose load	63.4 (33.8)	64.9 (38.4)	62.0 (28.3)
Homoeostasis model assessment insulin resistance	1.89 (0.71)	1.89 (0.79)	1.88 (0.64)
Lipids, mg/dL:			
Low density lipoprotein cholesterol	130.2 (29.1)	128.8 (26.2)	131.8 (31.6)
High density lipoprotein cholesterol	56.3 (17.6)	57.5 (14.2)	55.1 (20.3)
Triglycerides	140.1 (42.9)	139.5 (47.2)	140.7 (38.2)
Serum 25-hydroxyvitamin D, ng/mL	20.9 (6.1)	21.0 (6.2)	20.7 (6.1)
Distribution, No (%):			
<20 ng/mL	548 (43.6)	270 (42.9)	278 (44.4)
20-29 ng/mL	622 (49.5)	322 (51.1)	300 (47.9)
≥30 ng/mL	86 (6.8)	38 (6.0)	48 (7.7)
Serum 1,25-dihyroxyvitamin D, μg/dL	47.7 (22.2)	48.8 (23.5)	46.7 (20.8)

Primary outcome

During the three year follow-up period, 79 (12.5%) of 630 participants in the eldecalcitol group and 89 (14.2%) of 626 in the placebo group developed diabetes. We found no difference between treatment groups (hazard ratio for eldecalcitol versus placebo 0.87, 95% confidence interval 0.67 to 1.17; P=0.39) (fig 2, top panel).

Secondary outcomes

Similarly, we found no difference between treatment groups in regression to normoglycaemia. By the end of the study, 145 (23.0%) of 630 participants in the eldecalcitol group and 126 (20.1%) of 626 in the placebo group achieved normoglycaemia (hazard ratio 1.15, 0.93 to 1.41; P=0.21) (fig 3).

We did a multivariable Cox regression analysis after adjusting for 11 prespecified covariables (age, sex, hypertension, body mass index, family history of diabetes, glycated haemoglobin, fasting plasma glucose, two hour plasma glucose, 25-hydroxyvitamin D, HOMA-IR, and insulinogenic index) by using a multivariable fractional polynomial method. Figure 2 (bottom panel) shows the Kaplan-Meier curve after adjustment. We showed that eldecalcitol was effective for preventing the development of type 2 diabetes after adjustment for these covariables (hazard ratio 0.69, 0.51 to 0.95) at the significant level of P=0.020.

Sensitivity analysis

Sensitivity analyses for the per protocol population and complete population assessed the robustness of the primary outcome based on the intention-to-treat population. The result showed hazard ratios for eldecalcitol of 0.88 (0.65 to 1.19; P=0.39) and 0.88 (0.65 to 1.19; P=0.39) for the per protocol and complete populations, respectively, which were substantially the same as the primary analysis. In addition, the distributions of parameters related to glucose metabolism at the beginning of the study did not differ between the intention-to-treat and complete populations (supplementary table B).

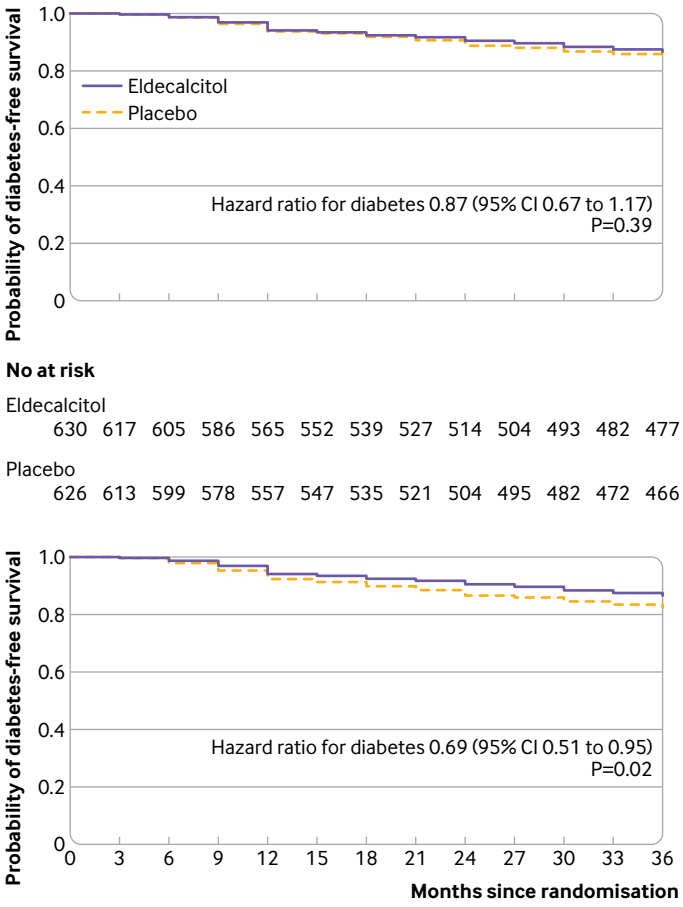

Fig 2 | Kaplan-Meier curves for survival free from type 2 diabetes among adults with impaired glucose tolerance. Top: before adjustment for 11 covariables. Bottom: after adjustment for 11 covariables

We investigated confounding covariables by using spline curve analysis one by one for 11 continuous covariables: age, body mass index, systolic blood pressure, glycated haemoglobin, fasting plasma glucose, fasting immunoreactive insulin, two hour plasma glucose, 25-hydroxyvitamin D, HOMA-IR, homoeostasis model assessment β cell function (HOMA-β), and insulinogenic index. We observed a confounding effect for glycated haemoglobin and two hour plasma glucose (supplementary figure A).

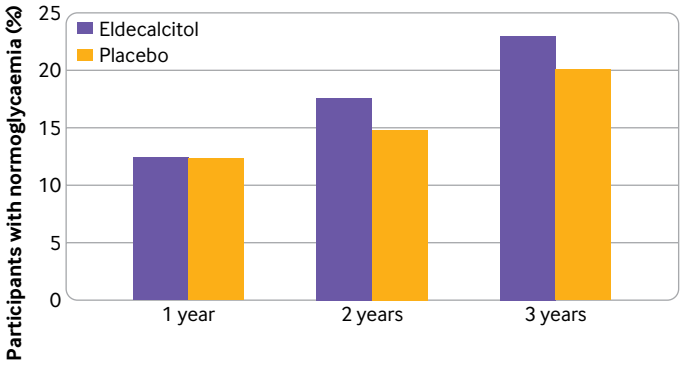

Fig 3 | Number of participants with impaired glucose tolerance who achieved normoglycaemia

Post hoc analysis

As a post hoc analysis, we re-examined the interactions of nine continuous covariables with eldecalcitol one by one after adjusting for glycated haemoglobin and two hour plasma glucose by using multivariable fractional polynomial Cox analysis. As shown in supplementary figure B, interactions with the eldecalcitol effect were seen for HOMA-β, HOMA-IR, and fasting immunoreactive insulin (HOMA-β=fasting immunoreactive insulin×360/fasting plasma glucose−63; HOMA-IR=fasting immunoreactive insulin×fasting plasma glucose/405) After we divided the three covariables into three groups at their 33.3 centile and 66.6 centile values, we did Kaplan-Meier analyses and multivariable fractional polynomial Cox regression. Supplementary figure C shows that eldecalcitol had a significant preventive effect on the development of type 2 diabetes among the lowest divisions of HOMA-β (hazard ratio 0.35, 0.21 to 0.59; P<0.001), HOMA-IR (0.37, 0.20 to 0.67; P=0.001), and fasting immunoreactive insulin (0.41, 0.23 to 0.71; P=0.001). These results indicate that eldecalcitol had a beneficial effect on insufficient basal insulin secretion.

Time trends of glycaemic status and body mass index

With regard to glycaemic status at the end of the trial, plasma glucose and immunoreactive insulin concentrations at fasting state and after 120 minutes did not differ between the two groups (fig 4). However, plasma glucose concentrations after 30 minutes of loading were significantly improved from those at baseline in the eldecalcitol group. We observed no significant differences in glycated haemoglobin and insulinogenic index between the two groups. HOMA-IR was significantly lower and HOMA-β was significantly higher in the eldecalcitol group than in the placebo group after the three year treatment period. Body mass index did not differ significantly between the two groups after treatment.

Time trends of bone metabolism markers

Although serum 25-hydroxyvitamin D concentration did not differ between the two groups, serum 1,25-dihydroxyvitamin D and bone alkaline phosphatase concentrations were significantly lower with eldecalcitol compared with placebo (fig 5). In addition, bone mineral densities of the lumbar spine and femoral neck were significantly higher with eldecalcitol than with placebo. Serum osteocalcin concentrations were significantly higher with eldecalcitol than with placebo. We found no significant differences between the two groups in serum leptin, receptor activator of nuclear factor-κ B ligand (RANKL), and osteoprotegerin concentrations.

Adverse events

A total of 26 (4.1%) participants in the eldecalcitol group and 21 (3.4%) in the placebo group discontinued the study owing to adverse events (hazard ratio 1.23, 0.70 to 2.16; P=0.47). Rates and types of adverse

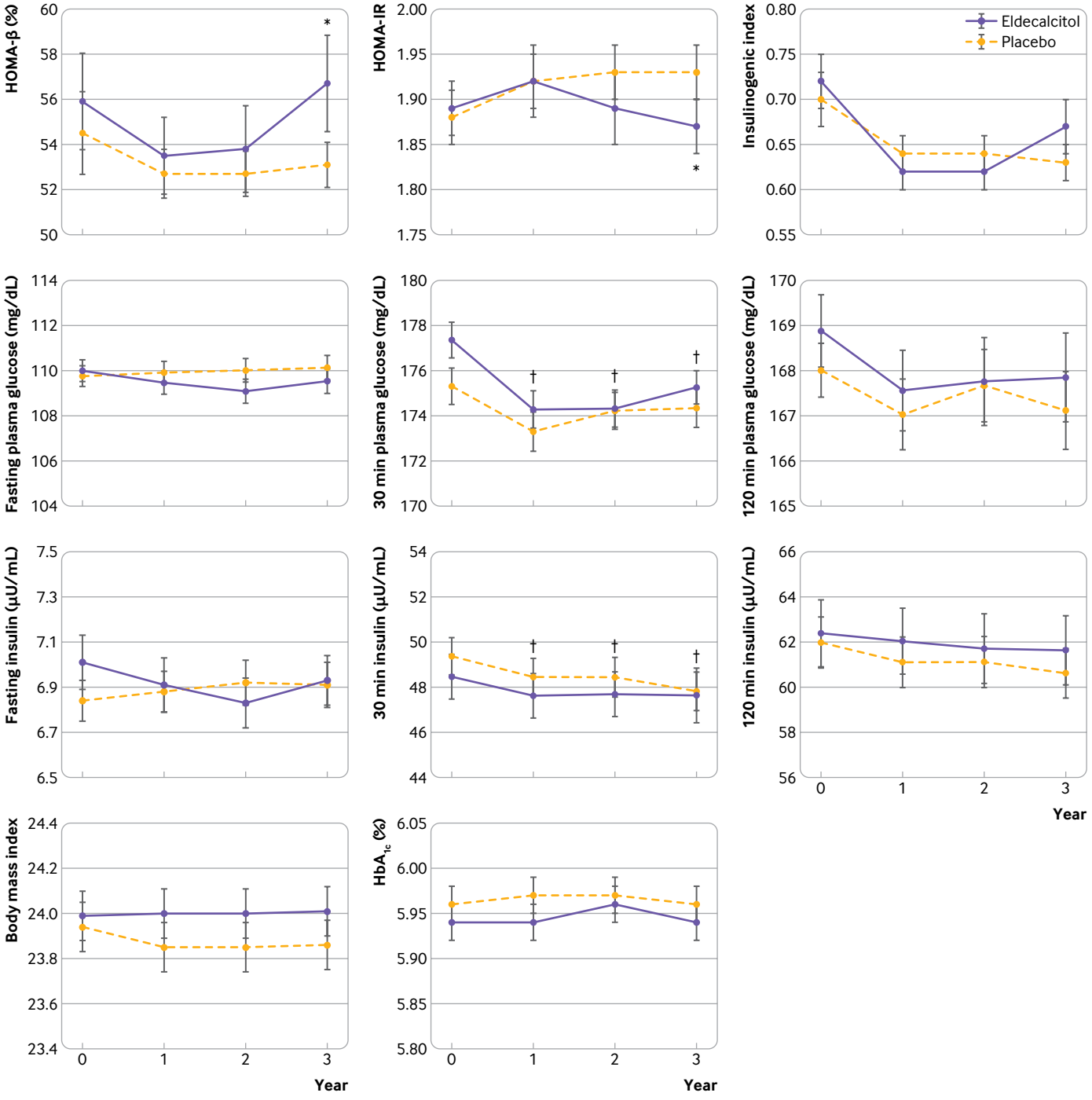

Fig 4 | Changes in glycaemic status and body mass index. Bars indicate standard error. HbA_{1c}=glycated haemoglobin; HOMA-β=homoeostasis model assessment β cell function; HOMA-IR=homoeostasis model assessment insulin resistance. *P<0.001 compared with placebo. †P<0.001 compared with baseline

events did not differ significantly between the two groups (table 2).

Discussion

In this DPVD trial, treatment with eldecalcitol (an active vitamin D analogue), at a dose of 0.75 μg per day, did not show a preventive effect on the incidence of type 2 diabetes, nor a beneficial effect on the rate of regression to normoglycaemia. However, we showed a preventive effect of eldecalcitol after adjusting for covariables (confounding factors) by using a multivariable fractional polynomial Cox regression analysis. The preventive effect of eldecalcitol on development of type 2 diabetes in a pre-diabetic population was seen especially among participants with insulin insufficiency. We believe that this discrepancy is a result of lack of statistical power, an unbalanced distribution of two hour plasma glucose concentrations between participants in the eldecalcitol and placebo groups, or both (supplementary figure D).

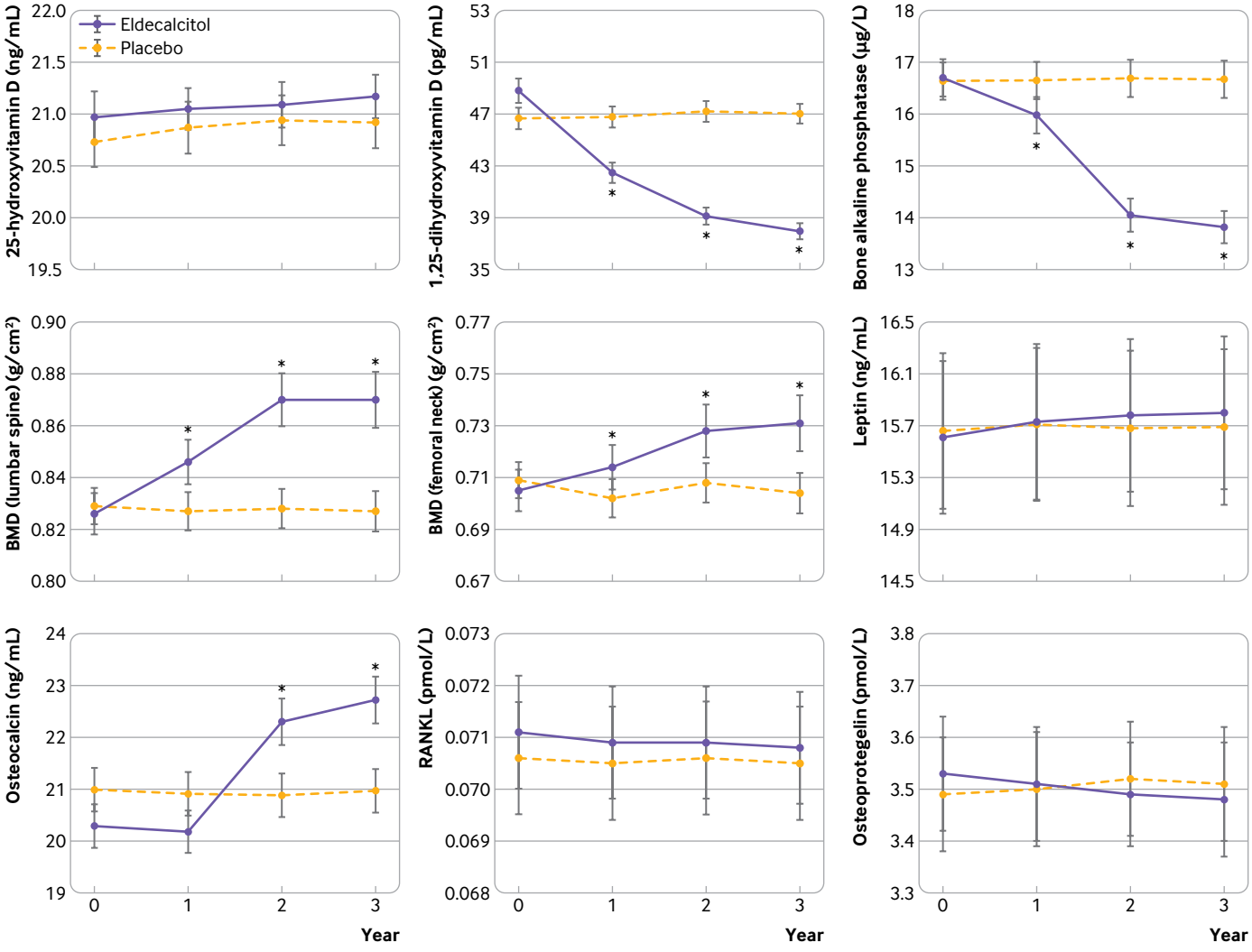

Fig 5 | Changes in bone metabolism markers. Bars indicate standard error. BMD=bone mineral density; RANKL=receptor activator of nuclear factor-κ B ligand. *P<0.001 compared with placebo

Firstly, we powered our trial to detect a 36% lower risk of diabetes with eldecalcitol than with placebo. However, eldecalcitol treatment decreased the risk of diabetes by a smaller effect size (13%). A meta-analysis published in 2020 showed that vitamin D supplementation was associated with an 11% lower risk of diabetes among 4896 patients with pre-diabetes.[18] Therefore, we suspect that our research was underpowered to detect the beneficial effect of eldecalcitol before adjustment for unbalanced covariables.

Glucose intolerance is associated with insufficient insulin secretion, insulin resistance, or both.[25] HOMA-κ is a biomarker of insulin secretion, with a value of less than 40% indicating decreased κ cell function.[37 38] HOMA-IR is a biomarker of insulin resistance, and a value of 1.6 or above indicates that insulin is secreted from the pancreas but the target organ's sensitivity to insulin is reduced and its action is slowed down—that is, insulin resistance.[39] Fasting immunoreactive insulin represents the amount of basal insulin; the normal range in Japan is 2-10 μU/mL,[25] but in Europe and the US it is 2.6-24.9 μU/mL,[40] which is a little higher. Eldecalcitol showed its beneficial effect among the participants with the lower third of HOMA-κ, HOMA-IR, and fasting immunoreactive insulin values, of which cut-off values were 44.0%, 1.49, and 5.6 μU/mL, respectively. This suggests that eldecalcitol might have a preventive effect for development of type 2 diabetes in pre-diabetic patients with impaired basal insulin secretion. Our results are backed by basic experiments and mouse genetics.[9 41]

As expected, the serum 25-hydroxyvitamin D concentration was not changed and the 1,25-dihydroxyvitamin D concentration was decreased by eldecalcitol treatment in this study. Eldecalcitol, an active vitamin D analogue, does not affect the serum 25-hydroxyvitamin D concentration, but it suppresses the expression of the *CYP27B1* gene in the kidney, which promotes conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D intracellularly.[42] In addition, it is relatively stable in the cell and strongly enhances the expression of the *CYP24* gene,[43] which suppresses 1,25-dihydroxyvitamin D production. As a result, the serum and intracellular 1,25-dihydroxyvitamin

Table 2 | Frequency of adverse events*

Events	Eldecalcitol (n=630)		Placebo (n=626)		Risk ratio (95%CI)
	No of events	Event rate: No/100 person years	No of events	Event rate: No/100 person years	
Discontinuations due to adverse events	26	1.38	21	1.12	1.23 (0.70 to 2.16)
Laboratory tests:					
Hypercalcaemia†	6	0.32	2	0.11	2.98 (0.60 to 14.71)
Hypercalciuria‡	5	0.27	3	0.16	1.66 (0.40 to 6.90)
Increased serum creatinine§	2	0.11	2	0.11	0.99 (0.14 to 7.03)
Nephrolithiasis	3	0.16	3	0.16	0.99 (0.20 to 4.90)
Hives	2	0.11	3	0.16	0.66 (0.11 to 3.95)
Digestive symptoms	3	0.16	2	0.11	1.49 (0.25 to 8.89)
Liver dysfunction	3	0.16	3	0.16	0.99 (0.20 to 4.90)
Death	1	0.05	1	0.05	0.99 (0.06 to 15.85)
Cancer	1	0.05	2	0.11	0.50 (0.05 to 5.47)
Serious adverse events	109	5.77	106	5.65	1.02 (0.80 to 1.30)
Respiratory system	34	1.80	37	1.97	0.91 (0.58 to 1.44)
Cardiovascular system	23	1.22	25	1.33	0.91 (0.53 to 1.59)
Gastrointestinal system	19	1.01	18	0.96	1.05 (0.56 to 1.98)
Urogenital system	15	0.79	12	0.64	1.24 (0.59 to 2.63)
Musculoskeletal system	14	0.74	11	0.59	1.27 (0.58 to 2.76)
Skin	4	0.21	3	0.16	1.33 (0.30 to 5.90)

*In participants who received at least one dose of eldecalcitol or placebo.
†Defined as corrected serum calcium >11.0 mg/dL (2.75 mmol/L), as confirmed on repeat testing.
‡Defined as fasting urine calcium:urine creatinine ratio ≥0.28.
§Defined as serum creatinine >1.5 mg/dL or upper limit of normal range for clinical laboratory at each clinical site.

D concentrations are decreased.[44 45] However, eldecalcitol has a similar physiological effect to 1,25-dihydroxyvitamin D. Hence, it increased the bone mineral densities of the lumbar spine and femoral neck in this study.

We also measured the changes in various factors (osteocalcin, leptin, RANKL, and osteoprotegerin) that are reportedly associated with glucose metabolism as well as bone metabolism after treatment with vitamin D and active vitamin D. Serum osteocalcin is a bone formation marker, which affects insulin sensitivity and glucose metabolism.[10-12 46] In the second year of the trial, serum osteocalcin concentrations began increasing significantly in the eldecalcitol group compared with the placebo group. Changes in serum osteocalcin concentrations by vitamin D supplementation were inconsistent in many previous studies.[47-49] This may have been due to relatively short trial durations, with participants not followed beyond three years. Our study also showed a decrease in 30 minute post-load plasma glucose concentrations and improvement of insulin resistance at three years by eldecalcitol. These results might be associated with changes in serum osteocalcin concentrations.

Leptin, secreted by adipose tissues, increases the sensation of satiety, and is involved in regulating food intake. Some trials have evaluated the effect of vitamin D supplementation on serum leptin concentrations in people with type 2 diabetic, but the results were inconsistent.[50-52] In our trial, serum leptin was slightly, but not significantly, elevated by eldecalcitol compared with placebo. Serum RANKL concentration, reported to be positively associated with insulin resistance,[53] showed a slight decrease in both groups, albeit with no significant difference. Serum osteoprotegerin, reportedly correlated with insulin sensitivity,[54 55] decreased slightly in the eldecalcitol group. However, we found no significant difference between the two groups.

We selected active vitamin D for this study for several reasons. Firstly, patients' adherence and continuation of the study will be high when active vitamin D is prescribed as a treatment.[56] Secondly, vitamin D supplements are abundant and ubiquitous in many foods and other sources, so simply evaluating vitamin D intake levels among participants in a trial is difficult. In contrast, active vitamin D is a medicine and is not contained in any food; thus, the drug intake can be easily evaluated. In addition, the effects with active vitamin D seem to be stronger than with normal vitamin D. Studies have shown that active vitamin D treatment increased the bone mineral density,[45 57 58] muscle strength,[59 60] and mobility of participants.[59 61] We also observed increased bone mineral density in this study. This finding might be associated with the improvement in participants' activity.

Strengths and limitations of study

Our trial has many strengths including a large population size, outpatient follow-up every three months, high rates of follow-up, and high adherence to the trial regimen. Moreover, this is the first published randomised controlled trial to assess the preventive effect of active vitamin D treatment on the development of type 2 diabetes in a pre-diabetic population and showed its beneficial effect on participants with insufficient insulin secretion. The mean serum 25-hydroxyvitamin D concentration at baseline was 20.9 ng/mL in our trial, which was lower than was obtained in previous studies (that is, 44.0 ng/mL and 28.0 ng/mL).[19 20] From the result shown in supplementary figure A, eldecalcitol may be more effective in patients with vitamin D deficiency than in patients without vitamin D deficiency.

The trial also had some limitations. Firstly, we used eldecalcitol at a regular dose of 0.75 µg. This is the standard dose administered in the case of osteoporosis, rickets, and hypocalcaemia in Japan. In studies in osteoporosis, a dose of 0.75 µg has shown non-inferiority in increasing bone mass and in preventing bone fracture compared with a higher dose (1 µg) and non-inferiority compared with a lower dose (0.5 µg) for the onset of adverse events.[44 58] However, whether it was an appropriate dose for prevention of diabetes in the context of this trial is unclear. Secondly, whether the results of this study apply to all ethnicities is unclear, because the study involved only Japanese participants. Latitude of living area, occupation, and racial or ethnic differences are important factors that affect serum 25-hydroxyvitamin D concentration.[62-64] Thirdly, the allocation method in this multicentre collaborative study may have been inadequate to prevent the imbalance of a critical variable such as baseline two hour plasma glucose concentration between the two groups. Therefore, a more sophisticated allocation method should be developed.

Conclusions

Treatment with eldecalcitol, an active vitamin D analogue, at a dose of 0.75 µg per day did not significantly reduce the incidence of diabetes and failed to increase the rate of regression to normoglycaemia compared with placebo among patients with impaired glucose tolerance who were at high risk for type 2 diabetes. Although our study suggested the potential for a beneficial effect of active vitamin D treatment on the prevention of type 2 diabetes after adjustment for confounding factors, this finding should be replicated in further populations before its significance for public health can be fully appreciated. Further research, such as an appropriately randomised study focused on pre-diabetic patients with insufficient basal insulin secretion or a meta-analysis including the results of this study, would be needed to determine whether vitamin D and/or active vitamin D is beneficial to people with pre-diabetes.

Contributors: TK wrote the first draft of the manuscript and was responsible for the design of the methods. TK, GS, SS, FK, and CK were responsible for the collection and assembly of data. TI was responsible for data management. SM completed the main part of the data analyses, and all authors discussed the analysis plan and results and provided input to the manuscript. All authors were responsible for the critical revision of the article for important intellectual content. All authors had access to the final study results and were responsible for the final approval of the manuscript. TK is the guarantor.

Funding: This study was supported by a grant from the Kitakyushu Medical Association. The sponsor did not contribute to the study design; the collection, management, analysis, or interpretation of data; the writing of the manuscript; or the decision to submit the manuscript for publication.

Competing interests: All authors have completed the ICMJE uniform disclosure form at https://www.icmje.org/disclosure-of-interest/ and declare: support by a grant from the Kitakyushu Medical Association; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Ethical approval: This trial was approved by the institutional review boards of Kokura Medical Association, University of Occupational and Environmental Health, and Fujisawa City Hospital. All participants gave written informed consent.

Data sharing: Relevant anonymised patient level data are available from the corresponding author on reasonable request.

The lead author (the manuscript's guarantor) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Provenance and peer review: Not commissioned; externally peer reviewed.

Dissemination to participants and related patient and public communities: The results of the study will be sent to research participants and research cooperation facilities via letters.

1 Ogurtsova K, da Rocha Fernandes JD, Huang Y, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 2017;128:40-50. doi:10.1016/j.diabres.2017.03.024
2 Huang Y, Cai X, Mai W, Li M, Hu Y. Association between prediabetes and risk of cardiovascular disease and all cause mortality: systematic review and meta-analysis. *BMJ* 2016;355:i5953. doi:10.1136/bmj.i5953
3 Tuomilehto J, Lindström J, Eriksson JG, et al, Finnish Diabetes Prevention Study Group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001;344:1343-50. doi:10.1056/NEJM200105033441801
4 Knowler WC, Barrett-Connor E, Fowler SE, et al, Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med* 2002;346:393-403. doi:10.1056/NEJMoa012512
5 Jackson L. Translating the Diabetes Prevention Program into practice: a review of community interventions. *Diabetes Educ* 2009;35:309-20. doi:10.1177/0145721708330153
6 Cardona-Morrell M, Rychetnik L, Morrell SL, Espinel PT, Bauman A. Reduction of diabetes risk in routine clinical practice: are physical activity and nutrition interventions feasible and are the outcomes from reference trials replicable? A systematic review and meta-analysis. *BMC Public Health* 2010;10:653. doi:10.1186/1471-2458-10-653
7 Johnson JA, Grande JP, Roche PC, Kumar R. Immunohistochemical localization of the 1,25(OH)2D3 receptor and calbindin D28k in human and rat pancreas. *Am J Physiol* 1994;267:E356-60.
8 Bland R, Markovic D, Hills CE, et al. Expression of 25-hydroxyvitamin D3-1alpha-hydroxylase in pancreatic islets. *J Steroid Biochem Mol Biol* 2004;89-90:121-5. doi:10.1016/j.jsbmb.2004.03.115
9 Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG. Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *FASEB J* 2003;17:509-11. doi:10.1096/fj.02-0424fje
10 Karsenty G, Olson EN. Bone and Muscle Endocrine Functions: Unexpected Paradigms of Inter-organ Communication. *Cell* 2016;164:1248-56. doi:10.1016/j.cell.2016.02.043
11 Tonks KT, White CP, Center JR, Samocha-Bonet D, Greenfield JR. Bone Turnover Is Suppressed in Insulin Resistance, Independent of Adiposity. *J Clin Endocrinol Metab* 2017;102:1112-21. doi:10.1210/jc.2016-3282
12 Conte C, Epstein S, Napoli N. Insulin resistance and bone: a biological partnership. *Acta Diabetol* 2018;55:305-14. doi:10.1007/s00592-018-1101-7
13 Song Y, Wang L, Pittas AG, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care* 2013;36:1422-8. doi:10.2337/dc12-0962
14 Afzal S, Bojesen SE, Nordestgaard BG. Low 25-hydroxyvitamin D and risk of type 2 diabetes: a prospective cohort study and metaanalysis. *Clin Chem* 2013;59:381-91. doi:10.1373/clinchem.2012.193003
15 von Hurst PR, Stonehouse W, Coad J. Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *Br J Nutr* 2010;103:549-55. doi:10.1017/S0007114509992017
16 Dutta D, Mondal SA, Choudhuri S, et al. Vitamin-D supplementation in prediabetes reduced progression to type 2 diabetes and was associated with decreased insulin resistance and systemic inflammation: an open label randomized prospective study from Eastern India. *Diabetes Res Clin Pract* 2014;103:e18-23. doi:10.1016/j.diabres.2013.12.044
17 Mitri J, Dawson-Hughes B, Hu FB, Pittas AG. Effects of vitamin D and calcium supplementation on pancreatic β cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *Am J Clin Nutr* 2011;94:486-94. doi:10.3945/ajcn.111.011684
18 Zhang Y, Tan H, Tang J, et al. Effects of Vitamin D Supplementation on Prevention of Type 2 Diabetes in Patients With Prediabetes: A Systematic Review and Meta-analysis. *Diabetes Care* 2020;43:1650-8. doi:10.2337/dc19-1708
19 Jorde R, Sollid ST, Svartberg J, et al. Vitamin D 20,000 IU per Week for Five Years Does Not Prevent Progression From Prediabetes to Diabetes. *J Clin Endocrinol Metab* 2016;101:1647-55. doi:10.1210/jc.2015-4013
20 Pittas AG, Dawson-Hughes B, Sheehan P, et al, D2d Research Group. Vitamin D Supplementation and Prevention of Type 2 Diabetes. *N Engl J Med* 2019;381:520-30. doi:10.1056/NEJMoa1900906
21 Seida JC, Mitri J, Colmers IN, et al. Clinical review: Effect of vitamin D3 supplementation on improving glucose homeostasis and preventing diabetes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2014;99:3551-60. doi:10.1210/jc.2014-2136
22 Poolsup N, Suksomboon N, Plordplong N. Effect of vitamin D supplementation on insulin resistance and glycaemic control in prediabetes: a systematic review and meta-analysis. *Diabet Med* 2016;33:290-9. doi:10.1111/dme.12893
23 Autier P, Mullie P, Macacu A, et al. Effect of vitamin D supplementation on non-skeletal disorders: a systematic review of meta-analyses and randomised trials. *Lancet Diabetes Endocrinol* 2017;5:986-1004. doi:10.1016/S2213-8587(17)30357-1
24 Kawahara T, Suzuki G, Inazu T, et al. Rationale and design of Diabetes Prevention with active Vitamin D (DPVD): a randomised, double-blind, placebo-controlled study. *BMJ Open* 2016;6:e011183. doi:10.1136/bmjopen-2016-011183

25 Seino Y, Nanjo K, Tajima N, et al, Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig* 2010;1:212-28. doi:10.1111/j.2040-1124.2010.00074.x
26 International Diabetes Federation. *IDF Diabetes Atlas.* IDF, 2013.
27 American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010;33(Suppl 1):S62-9. doi:10.2337/dc10-S062
28 Royston P, Sauerbrei W. A new approach to modelling interactions between treatment and continuous covariates in clinical trials by using fractional polynomials. *Stat Med* 2004;23:2509-25. doi:10.1002/sim.1815
29 Wood SN. *Generalized additive models: an introduction with R.* 2nd ed. Chapman & Hall/CRC, 2017. doi:10.1201/9781315370279.
30 Rubin DB, Schenker N. Multiple imputation in health-care databases: an overview and some applications. *Stat Med* 1991;10:585-98. doi:10.1002/sim.4780100410
31 van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. *J Stat Softw* 2011;45:1-67. doi:10.18637/jss.v045.i03.
32 Little DJA, Rubin NB. *Statistical analysis with missing data.* Hoboken, 2020.
33 Sterne JA, White IR, Carlin JB, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 2009;338:b2393. doi:10.1136/bmj.b2393
34 Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;84:18-28. doi:10.1093/ajcn/84.1.18
35 Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-81. doi:10.1056/NEJMra070553
36 Okazaki R, Ozono K, Fukumoto S, et al. Assessment criteria for vitamin D deficiency/insufficiency in Japan - proposal by an expert panel supported by Research Program of Intractable Diseases, Ministry of Health, Labour and Welfare, Japan, The Japanese Society for Bone and Mineral Research and The Japan Endocrine Society [Opinion][Opinion]. *Endocr J* 2017;64:1-6. doi:10.1507/endocrj.EJ16-0548
37 Takita M, Matusmoto S. SUITO index for evaluation of clinical islet transplantation. *Cell Transplant* 2012;21:1341-7. doi:10.3727/096368912X636885
38 Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004;27:1487-95. doi:10.2337/diacare.27.6.1487
39 Gayoso-Diz P, Otero-González A, Rodriguez-Alvarez MX, et al. Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord* 2013;13:47. doi:10.1186/1472-6823-13-47
40 Rifai N, Horvath AR, Wittwer C, et al. *Tietz textbook of clinical chemistry and molecular diagnostics* . 6th edition. Elsevier, 2018.
41 Berridge MJ. Vitamin D deficiency and diabetes. *Biochem J* 2017;474:1321-32. doi:10.1042/BCJ20170042
42 Ono Y. Multifunctional and potent roles of the 3-hydroxypropoxy group provide eldecalcitol's benefit in osteoporosis treatment. *J Steroid Biochem Mol Biol* 2014;139:88-97. doi:10.1016/j.jsbmb.2013.10.007
43 Ritter CS, Brown AJ. Suppression of PTH by the vitamin D analog eldecalcitol is modulated by its high affinity for the serum vitamin D-binding protein and resistance to metabolism. *J Cell Biochem* 2011;112:1348-52. doi:10.1002/jcb.23051
44 Matsumoto T, Takano T, Yamakido S, Takahashi F, Tsuji N. Comparison of the effects of eldecalcitol and alfacalcidol on bone and calcium metabolism. *J Steroid Biochem Mol Biol* 2010;121:261-4. doi:10.1016/j.jsbmb.2010.03.035
45 Matsumoto T, Ito M, Hayashi Y, et al. A new active vitamin D3 analog, eldecalcitol, prevents the risk of osteoporotic fractures--a randomized, active comparator, double-blind study. *Bone* 2011;49:605-12. doi:10.1016/j.bone.2011.07.011
46 Wang CM, Chang CS, Chang YF, et al. Inverse Relationship between Metabolic Syndrome and 25-Hydroxyvitamin D Concentration in Elderly People without Vitamin D deficiency. *Sci Rep* 2018;8:17052. doi:10.1038/s41598-018-35229-2
47 Rahme M, Sharara SL, Baddoura R, et al. Impact of Calcium and Two Doses of Vitamin D on Bone Metabolism in the Elderly: A Randomized Controlled Trial. *J Bone Miner Res* 2017;32:1486-95. doi:10.1002/jbmr.3122
48 Schwetz V, Trummer C, Pandis M, et al. Effects of Vitamin D Supplementation on Bone Turnover Markers: A Randomized Controlled Trial. *Nutrients* 2017;9:623. doi:10.3390/nu9050432
49 Castle M, Fiedler N, Pop LC, et al. Three Doses of Vitamin D and Cognitive Outcomes in Older Women: A Double-Blind Randomized Controlled Trial. *J Gerontol A Biol Sci Med Sci* 2020;75:835-42. doi:10.1093/gerona/glz041
50 Tabesh M, Azadbakht L, Faghihimani E, Tabesh M, Esmaillzadeh A. Calcium-vitamin D cosupplementation influences circulating inflammatory biomarkers and adipocytokines in vitamin D-insufficient diabetics: a randomized controlled clinical trial. *J Clin Endocrinol Metab* 2014;99:E2485-93. doi:10.1210/jc.2014-1977
51 Breslavsky A, Frand J, Matas Z, Boaz M, Barnea Z, Shargorodsky M. Effect of high doses of vitamin D on arterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients. *Clin Nutr* 2013;32:970-5. doi:10.1016/j.clnu.2013.01.020
52 Ghavamzadeh S, Mobasseri M, Mahdavi R. The Effect of Vitamin D Supplementation on Adiposity, Blood Glycated Hemoglobin, Serum Leptin and Tumor Necrosis Factor-α in Type 2 Diabetic Patients. *Int J Prev Med* 2014;5:1091-8.
53 Kiechl S, Wittmann J, Giaccari A, et al. Blockade of receptor activator of nuclear factor-κB (RANKL) signaling improves hepatic insulin resistance and prevents development of diabetes mellitus. *Nat Med* 2013;19:358-63. doi:10.1038/nm.3084
54 Ashley DT, O'Sullivan EP, Davenport C, et al. Similar to adiponectin, serum levels of osteoprotegerin are associated with obesity in healthy subjects. *Metabolism* 2011;60:994-1000. doi:10.1016/j.metabol.2010.10.001
55 Pérez de Ciriza C, Lawrie A, Varo N. Osteoprotegerin in Cardiometabolic Disorders. *Int J Endocrinol* 2015;2015:564934. doi:10.1155/2015/564934
56 Pilz S, Rutters F, Dekker JM. Disease prevention: vitamin D trials. *Science* 2012;338:883. doi:10.1126/science.338.6109.883-c
57 Papadimitropoulos E, Wells G, Shea B, et al, Osteoporosis Methodology Group and The Osteoporosis Research Advisory Group. Meta-analyses of therapies for postmenopausal osteoporosis. VIII: Meta-analysis of the efficacy of vitamin D treatment in preventing osteoporosis in postmenopausal women. *Endocr Rev* 2002;23:560-9. doi:10.1210/er.2001-8002
58 Matsumoto T, Miki T, Hagino H, et al. A new active vitamin D, ED-71, increases bone mass in osteoporotic patients under vitamin D supplementation: a randomized, double-blind, placebo-controlled clinical trial. *J Clin Endocrinol Metab* 2005;90:5031-6. doi:10.1210/jc.2004-2552
59 Iwamoto J, Sato Y. Eldecalcitol improves chair-rising time in postmenopausal osteoporotic women treated with bisphosphonates. *Ther Clin Risk Manag* 2014;10:51-9. doi:10.2147/TCRM.S54772
60 Beaudart C, Buckinx F, Rabenda V, et al. The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab* 2014;99:4336-45. doi:10.1210/jc.2014-1742
61 Rosendahl-Riise H, Spielau U, Ranhoff AH, Gudbrandsen OA, Dierkes J. Vitamin D supplementation and its influence on muscle strength and mobility in community-dwelling older persons: a systematic review and meta-analysis. *J Hum Nutr Diet* 2017;30:3-15. doi:10.1111/jhn.12394
62 Asakura K, Etoh N, Imamura H, et al. Vitamin D Status in Japanese Adults: Relationship of Serum 25-Hydroxyvitamin D with Simultaneously Measured Dietary Vitamin D Intake and Ultraviolet Ray Exposure. *Nutrients* 2020;12:743. doi:10.3390/nu12030743
63 Hiraki LT, Major JM, Chen C, et al. Exploring the genetic architecture of circulating 25-hydroxyvitamin D. *Genet Epidemiol* 2013;37:92-8. doi:10.1002/gepi.21694
64 Jiang X, O'Reilly PF, Aschard H, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. *Nat Commun* 2018;9:260. doi:10.1038/s41467-017-02662-2

Web appendix: Supplementary materials