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ORIGINAL ARTICLE

The effect of high-dose vitamin D_3 supplementation on bone mineral density in subjects with prediabetes

A. U. Larsen[1] · G. Grimnes[1,2] · R. Jorde[1,2]

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Abstract
Summary The rationale of this study was to determine the effect of high-dose vitamin D_3 supplementation on bone mineral density (BMD). Prediabetic males given vitamin D had significantly less reduction in BMD at the femoral neck compared to the controls. The clinical implications of our findings require further investigation.
Introduction Type 2 diabetes mellitus is associated with increased fracture risk, and recent studies show crosstalk between bone and glucose metabolism. Few studies have investigated the effect of vitamin D supplementation on the bone without additional calcium. In the present study, we aimed to determine whether a high dose of vitamin D_3 could improve bone mass density (BMD) in prediabetic subjects.
Methods The current study was conducted as a secondary research on a previously performed trial, in which 511 subjects with prediabetes were randomized to vitamin D_3 (20,000 IU per week) versus placebo for 5 years. BMD was measured using dual-energy X-ray absorptiometry (DEXA).
Results Two hundred and fifty-six subjects were randomized to vitamin D and 255 to placebo. Mean baseline serum 25-hydroxyvitamin D (25(OH)D) level was 60 nmol/L. Two hundred and two and 214 in the vitamin D and placebo groups, respectively, completed BMD measurements, whereas one in each group was excluded due to use of bisphosphonates. Males given vitamin D had significantly less reduction in BMD at the femoral neck measurement site compared to the controls (0.000 versus − 0.010 g/cm^2, $p = 0.008$). No significant differences between intervention groups were seen at the total hip measurement site, regarding both males and females.
Conclusions Vitamin D_3 supplementation alone may be beneficial in males with prediabetes, but confirmatory studies are needed.

Keywords Bone mineral density · Prediabetes · Randomized controlled trial · Vitamin D

Introduction

Diabetes mellitus is one of the world's most common chronic diseases, and overall prevalence among adults is estimated to increase in years to come [1, 2]. Blood glucose is, however, continuous, and type 2 diabetes mellitus (T2DM) develops through a prediabetic stage, defined by impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT) [3, 4]. Such modest disturbances of glucose metabolism may increase the risk of complications traditionally attributed to T2DM, such as retinopathy, nephropathy, myocardial infarctions, and stroke, and both macro and microvascular damage appear to precede the onset of overt disease [5, 6].

Recently, it has been argued that the effects of chronically elevated glucose levels on the bone should be added to the more well-known complications of inadequately regulated glucose metabolism [7]. This is in line with the growing evidence of increased fracture risk in patients with T2DM,

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✉ A. U. Larsen
anette.uhlving@post.uit.no

[1] Tromsø Endocrine Research Group, Department of Clinical Medicine, UiT The Arctic University of Norway, 9037 Tromsø, Norway

[2] Division of Internal Medicine, University Hospital of North Norway, 9038 Tromsø, Norway

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although these individuals are reported to have higher bone mineral density (BMD) than non-diabetic subjects [8–10]. It has been hypothesized that the accumulation of advanced glycation end products, impaired bone healing, and altered body composition, as well as an increased production of non-enzymatic cross-links within collagen fibers, have a negative impact on bone matrix properties [7]. Despite these findings, a recent meta-analysis exploring correlations of abnormal glucose metabolism reported no significant correlations neither with BMD nor with bone metabolism [11]. However, the increased propensity to fractures in patients with abnormal glucose metabolism may be caused by less apparent qualitative changes [12]. The notion of the bone being a true endocrine organ and an important regulator of whole-body glucose metabolism [13, 14] further complicates the relationship. In any case, improved bone health would be considered beneficial.

Vitamin D deficiency has been linked to both high blood glucose levels, insulin resistance, and greater risk of developing T2DM, although so far, the results of large RCTs do not support a causal relationship [15]. The role of vitamin D in maintenance of a healthy, mineralized skeleton through regulation of calcium and phosphate homeostasis is, however, well known. Moreover, vitamin D may contribute to improved bone health independent of its role in calcium homeostasis.

The active metabolite, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), has been suggested to exert local autocrine and paracrine regulation of bone turnover, in which $1,25(OH)_2D$ can stimulate both bone formation and resorption [16, 17]. Locally produced $1,25(OH)_2D$ is important for an optimized communication and coupling mechanism between osteoblasts and osteoclasts [18], as well as in osteoblast differentiation of human bone marrow cells [19, 20]. Moreover, $1,25(OH)_2D$ seems to affect secretion of osteoprotegerin from mature osteoblasts [21], and both the vitamin D receptor and the enzyme necessary for activation of 25(OH)D to $1,25(OH)_2D$, CYP27B1 (1-alphahydroxylase), are present in bone cells [16]. However, vitamin D may directly inhibit mineralization of the bone through increased local pyrophosphate concentrations [22], and the vitamin D-induced secretion of osteoprotegerin from osteoblasts has, together with RANKL, been suggested to stimulate osteoclastogenesis, thereby increasing bone resorption [23]. The latter also applies in states of vitamin D deficiency where secondary hyperparathyroidism arises, followed by a stimulated production of RANKL and osteoclastogenesis. Thus, vitamin D may exert biphasic effects, although consensus regarding this matter is yet to be reached.

In the present study, we hypothesized that supplementation with vitamin D could increase BMD in subjects with prediabetes, and thereby exert a preventive effect on fracture risk in this potentially exposed group.

Methods

Study design

The design of the study, where the main intention was to evaluate vitamin D for the prevention of T2DM, has been described in detail before [15, 24]. In short, prediabetic subjects (IFG (fasting serum glucose 6.0–6.9 mmol/L) and/or IGT (fasting serum glucose < 7.0 mmol/L and 2-h value 7.8–11.0 mmol/L at oral glucose tolerance test (OGTT) with 75 g glucose)) were included. Subjects were of both sexes, aged 25–80 years old. Most of them were recruited after participation in the sixth survey of the Tromsø Study (2007–2008) where 4393 subjects with hemoglobin A_{1c} (HbA_{1c}) in the range 5.0–6.9% (39.9–51.9 mmol/mol) and not previously diagnosed with diabetes, were invited to an OGTT, which was completed in 3476 subjects. Among these, 713 had IFG and/or IGT and were invited by letter to participate in the present study. In addition, a few other subjects were invited based on follow-up OGTTs performed in participants in previous studies [25, 26]. Subjects with primary hyperparathyroidism, granulomatous disease, history of urolithiasis, cancer diagnosed in the past 5 years, unstable angina pectoris, myocardial infarction, or stroke in the past year were excluded. Pregnant or lactating women, or women of fertile age with no use of contraception, were not included.

At the first visit, a brief clinical examination was performed, and questionnaires were filled in. The latter included questions on medical history and use of dietary supplementations. Height and weight were measured wearing light clothing. Fasting blood samples had been collected at the OGTT, and supplementary non-fasting blood samples were drawn at this first visit in the study. In all subjects, BMD was measured at baseline and at their last visit in the study with dual-energy X-ray absorptiometry (DEXA) (GE Lunar Prodigy, Lunar Corporation, Madison, WI, USA) at the femoral neck and total hip measurement site. The scanner was calibrated daily against the standard calibration block supplied by the manufacturer (aluminum spine phantom), and these measurements showed no drift throughout the study. The subjects were then randomized (non-stratified) in a 1:1 ratio to one capsule vitamin D_3 (cholecalciferol 20,000 IU per week (Dekristol; Mibe, Jena, Germany)) or an identical-looking placebo capsule containing arachis oil (Hasco-Lek, Wroclaw, Poland). New medication was supplied every sixth month, and unused capsules were returned and counted. The subjects were instructed not to take vitamin D supplements (including cod liver oil) exceeding 400 IU per day during the study.

For the next 5 years, the subjects met annually for new OGTTs, supplemental serum sampling, and height, weight, and blood pressure measurements. As part of a safety monitoring, serum calcium and creatinine were measured every sixth month, in between annual visits. At the annual visits,

all subjects were asked to fill in the same questionnaires as at the baseline visit. Adverse events were specifically asked for at all visits.

If at the annual OGTT the fasting blood glucose was > 6.9 mmol/L and/or the 2-h value > 11.0 mmol/L, the subject was considered to have T2DM, thus ending their participation in the study. These subjects were thereafter retested (if necessary) and followed by their general practitioner. HbA_{1c} was implemented in the present study as a diagnostic criterion from November 2012 [15], and thereafter, subjects were retested with a new HbA_{1c} measurement if HbA_{1c} alone was ≥ 6.5%. If still ≥ 6.5% after retest, subjects were diagnosed with T2DM, thereby ending their participation. Also, if diagnosed elsewhere with T2DM in between visits, participation in the study was ended.

Subjects with persistent measurements of serum calcium > 2.55 mmol/L were excluded, as well as subjects who developed renal stones, or symptoms compatible with renal stones. In the initial protocol, subjects who during the study were diagnosed with cancer, coronary infarction, unstable angina pectoris, or stroke were to be excluded from the study. From October 2011, this was changed to exclusion of subjects who during the study developed serious disease making it difficult or impossible to attend scheduled visits.

Biochemical analyses including serum 25(OH)D were analyzed using the gold standard LC-MSMS method, as previously described [15].

Statistical analyses

Normal distribution was evaluated by visual inspection of histograms, and by kurtosis and skewness. Log transformation was performed where appropriate. Comparisons of intervention groups at baseline were performed with Student's *t* test for continuous variables, Pearson's chi-square test for categorical variables, and Mann-Whitney *U* test for variables with a non-normal distribution. For BMD, the mean value of left and right measures was used for statistical analyses (when both values could be obtained). If only one side could be measured, this value was chosen to represent the mean value. Initially, measurements were to be classified as normal if corresponding to a T-score ≥ − 1.0, and if corresponding to a T-score between − 1.0 and − 2.5 or ≤ − 2.5 as osteopenic or osteoporotic, respectively [27]. However, since no male subjects and only very few female subjects presented with osteoporotic T-scores, all subjects with T-scores < − 1.0 were classified as osteopenic. Participants reporting use of bisphosphonates during the study were excluded from all statistical analyses. Predictors of baseline BMD were evaluated with multiple linear regression, applying forced entry on all predictor variables. Regarding change in BMD (delta values calculated as BMD at the last visit in the study minus BMD at baseline), comparison of the vitamin D and the placebo group was done using Student's *t* test. If significant, change in BMD was further tested with a linear regression model adjusting for baseline values [28], observation time and variables significantly predicting BMD at baseline (Table 2). All subgroups were analyzed likewise. The incidence of fractures during the study in the vitamin D and the placebo group was tested with a binary logistic regression analysis, adjusted for age and BMI.

A power calculation was made for the main endpoint (development of T2DM) [15], but a separate power calculation regarding BMD was not made. All tests were done two-sided, and $p < 0.05$ was considered statistically significant.

Statistical analyses were performed per protocol, using SPSS software version 24 (IBM Corp, Chicago, IL).

Ethics

Written informed consent for participation in the study was provided by all subjects who accepted the invitation. The study was approved by the Regional Committee for Medical Research Ethics (REK NORD 81/2007) and by the Norwegian Medicines Agency (2007-002167-27). The trial is registered at ClinicalTrials.gov (NCT00685594).

Results

A total of 511 subjects were included in the main study on prevention of T2DM. Ninety-five subjects were excluded due to missing baseline and/or final BMD measurements, and an additional two subjects were excluded due to use of bisphosphonates, thus leaving 414 subjects (201 given vitamin D and 213 given placebo) for the BMD analyses in the present study. Among these, 111 in the vitamin D group and 109 in the placebo group completed the 5-year intervention period. The flow of the study is shown in Fig. 1. Median observation time was 59 months in both of the male intervention groups ($p = 0.738$), while a non-significant difference in observation time was found between the female intervention groups with a median of 59 months in the vitamin D group versus 48 months in the placebo group ($p = 0.177$).

Baseline characteristics of the study participants are shown in Table 1, and no significant differences between the vitamin D and the placebo group were observed. The baseline serum 25(OH)D levels were 59.7 ± 22.0 nmol/L in the vitamin D group and 61.5 ± 20.4 nmol/L in the placebo group. During the 5-year intervention, mean serum 25(OH)D levels in the vitamin D group increased to 114.7 ± 27.4 nmol/L, whereas only minor changes were observed in mean serum 25(OH)D levels in the placebo group, as shown for males in Figs. 2 and 3. After 1 year, median serum PTH fell from 5.3 ± 2.1 to 5.0 ± 1.8 pmol/L in the vitamin D group, in contrast to an increase from 5.1 ± 2.1 to 5.2 ± 2.2 pmol/L in the placebo group ($p = 0.005$). A similar difference persisted throughout

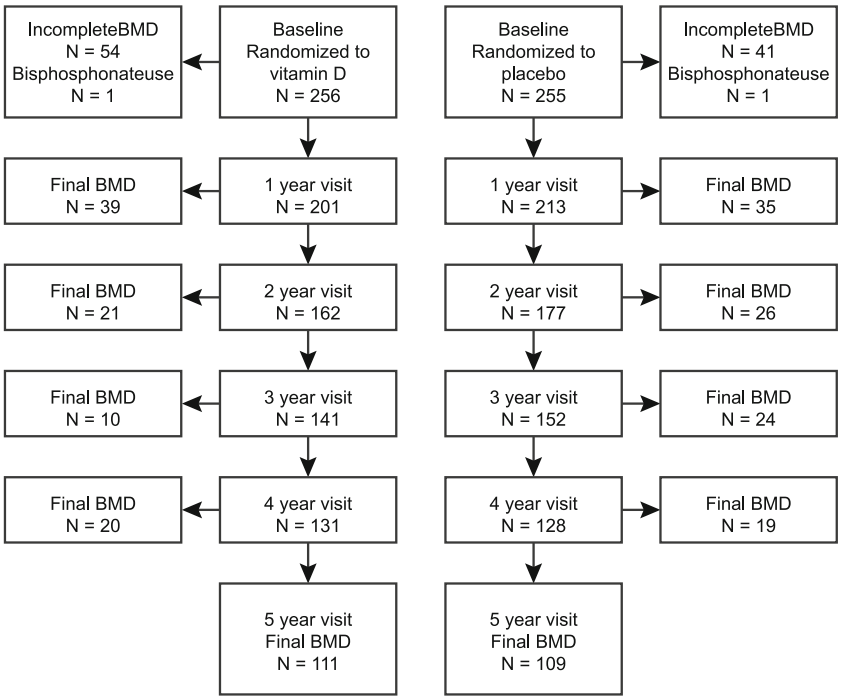

Fig. 1 Flowchart of the study

the study, both in men and women. The compliance rate was between 95 and 99% at all visits in both groups.

The baseline characteristics of the 97 subjects excluded due to missing BMD measurements can be found in Supplemental Table 1. Among these, there were no significant differences between the 55 subjects given vitamin D and the 42 subjects given placebo, nor were there any significant differences between the included (414 subjects) and the excluded (97 subjects) at baseline.

Among the entire study population, a total of 3885 adverse events were recorded during the 5-year intervention period, with no significant differences between intervention groups. Adverse events and side effects, including serious and/or calcium-specific events, have been described in detail before, and no serious side effects related to the intervention were recorded [15]. In the present study, we looked specifically at incident fractures. A total of 22 fractures were recorded among the subjects with valid BMD measurements, of which nine were in men. Of these nine, three fractures were recorded in the vitamin D group, against six in the placebo group. There was no significant difference in the number of fractures between the vitamin D group and the placebo group (adjusting for age, weight, and height); neither in general ($p = 0.868$) nor in stratified analyses (males, $p = 0.384$ versus females, $p = 0.249$).

BMD measurements

There was a non-significant trend ($p = 0.06$) for interaction between gender and treatment versus BMD at the femoral neck site, and thus, we chose to compare intervention groups regarding change in mean BMD separately for men and women. Body mass index (BMI) and tobacco use were found to significantly predict baseline BMD at the femoral neck and total hip measurement site in both sexes. Age significantly predicted baseline BMD at the femoral neck and total hip in females, whereas predicting baseline BMD only at the femoral neck measurement site in males. Additionally, baseline BMD in males was significantly predicted by serum calcium, PTH, and creatinine at both measurement sites (Table 2). There were no statistically significant differences in baseline BMD in the vitamin D and placebo group neither at the femoral neck, nor at the total hip (Table 3).

In males given vitamin D, there was no reduction in BMD at the femoral neck from baseline to the last visit in the study, values being 0.974 g/cm^2 at both visits respectively (Table 3). With adjustment for baseline BMD, observation time, and statistically significant predictors of baseline BMD (Table 2), this change differed significantly ($p = 0.008$) from that in the placebo group, of which corresponding values were 0.984 g/cm^2 at baseline and 0.973 g/cm^2 at the final visit (Table 3). At the total hip measurement site, a marginal difference was found between males given vitamin D versus placebo (an increase from 1.063 g/cm^2 at baseline to 1.065 g/cm^2 at final measurement in the vitamin D group versus a reduction from 1.078 to 1.075 g/cm^2 in the placebo group). However, this difference did not reach statistical significance ($p = 0.130$).

Regarding females, no significant differences were found between the two groups at either measurement site (Table 3).

Table 1 Baseline characteristics of the 414 study subjects

Variables	Males			Females		
	Vitamin D group (n = 125)	Placebo group (n = 131)	p value	Vitamin D group (n = 76)	Placebo group (n = 82)	p value
Age (years)	61.1 ± 7.6	61.0 ± 8.8	0.980	62.8 ± 8.3	63.1 ± 9.2	0.841
BMI (kg/m^2)	30.0 ± 3.8	30.1 ± 4.4	0.813	30.1 ± 4.3	29.4 ± 4.7	0.311
Tobacco use (%)	24.8	19.1	0.269	18.2	15.9	0.668
Femoral neck BMD (g/cm^2)	0.974 ± 0.126	0.984 ± 0.136	0.561	0.918 ± 0.117	0.887 ± 0.137	0.137
Total hip BMD (g/cm^2)	1.063 ± 0.137	1.078 ± 0.133	0.393	1.003 ± 0.129	0.961 ± 0.140	0.055
Osteopenia femoral neck (%)	4.8	6.1	0.646	5.3	8.5	0.419
Osteopenia total hip (%)	12.0	14.5	0.555	17.1	12.2	0.382
Serum 25(OH)D (nmol/L)	58.5 ± 23.0	59.0 ± 18.3	0.860	61.7 ± 20.3	65.7 ± 23.0	0.258
Vitamin D supplement use[a] (%)	30.4	38.9	0.152	36.8	28.0	0.238
Serum calcium, mmol/L	2.31 ± 0.07	2.30 ± 0.08	0.239	2.31 ± 0.07	2.32 ± 0.10	0.573
Calcium supplement use (%)	3.2	6.1	0.271	15.8	24.4	0.179
Serum PTH[b] (pmol/L)	5.3 (2.2)	5.2 (2.3)	0.443	5.7 (2.2)	5.2 (2.9)	0.514
Serum creatinine (μmol/L)	74.3 ± 12.9	75.4 ± 12.3	0.504	61.0 ± 9.7	61.1 ± 10.8	0.947
HbA_{1c} (%)	6.0 ± 0.3	5.9 ± 0.3	0.097	6.0 ± 0.3	6.0 ± 0.4	0.374
HbA_{1c} (mmol/mol)	42.0 ± 3.0	41.0 ± 3.0	–	42.0 ± 3.0	42.0 ± 4.0	–

Numbers represent mean ± SD, unless otherwise specified. Osteopenia T-score < −1.0

BMI body mass index, *BMD* bone mass density, *25(OH)D* 25-hydroxyvitamin D, *PTH* parathyroid hormone, *HbA_{1c}* hemoglobin A_{1c}

[a] Including cod liver oil

[b] Non-normal distribution, numbers represent median (IQR)

Subgroup analyses

A subgroup analysis was performed to investigate whether a more pronounced effect of vitamin D on BMD could be detected if including only subjects with 25(OH)D levels below 50 nmol/L. Thus, 68 subjects (47 males) in the vitamin D group and 63 (40 males) in the placebo group had serum 25(OH)D levels < 50 nmol/L at baseline (Supplemental Table 2). There were no significant differences between the intervention groups at baseline, and although the same trend was observed, with a marginal increase in BMD during the study among the males given vitamin D (data not shown), the difference versus the placebo group did not reach statistical significance (p = 0.072).

Due to the unique opportunity of investigating the effect of vitamin D supplementation on BMD without any supplemental dietary calcium, an additional subgroup analysis was performed, excluding users of calcium supplements at baseline and during the study. This analysis rendered 177 subjects (116 males) in the vitamin D group and 177 subjects (118 males) in the placebo group. The two groups were similar at baseline (Supplemental Table 3), and statistical regression analyses rendered the same results as in the main analyses regarding predictors of baseline mean BMD. Also, a statistically significant interaction term persisted between gender and intervention (p = 0.048), and stratified linear regression analyses produced the same results as when calcium users were included, with a statistically significant change in BMD at the femoral neck in men (p = 0.019), but not at other measurement sites and with no significant effects in women (data not shown).

Moreover, to investigate whether the difference between the vitamin D and the placebo group differed depending on their prediabetes-diagnosis, the cohort was split into three groups including those with (1) elevated fasting blood glucose only (6.0–6.9 mmol/L), (2) elevated 2-h values only (7.8–11.0 mmol/L), and (3) elevated measurements of both fasting and 2-h values of blood glucose. The sub-cohorts were then analyzed separately (applying the same statistical methods as in the main analyses) comparing delta BMD at the femoral neck and total hip in the vitamin D versus the placebo group. However, as the results were non-significant, these data are not shown.

Finally, subgroup analyses including only subjects with T-scores < − 1.0 were also carried out; however, few subjects were eligible for such analyses (Table 1), and no significant effects were detected at either measurement site (data not shown).

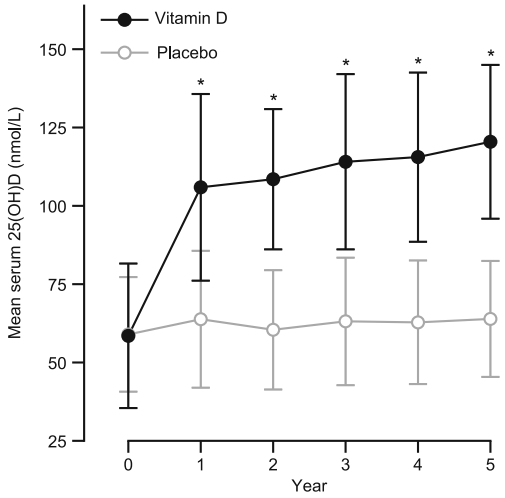

Fig. 2 Mean serum 25(OH)D levels during the study in the 125 males in the vitamin D group and the 131 males in the placebo group. Error bars represent 1 SD. Asterisks indicate $p < 0.001$ versus the control group with Student's *t* test

Discussion

In the present study, we hypothesized that supplementation with vitamin D could increase BMD in subjects with prediabetes, and we found a small, but significant positive effect of vitamin D supplementation on femoral neck BMD in males. To our knowledge, this has not been shown before. At the total hip measurement site, a positive, but non-significant effect was found. In the females, the vitamin D and the placebo group did not differ significantly.

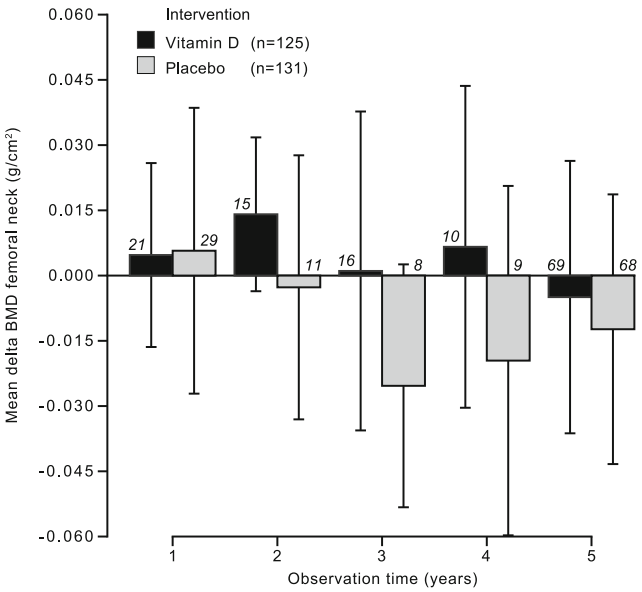

Fig. 3 Mean delta BMD (calculated as BMD at the last visit in the study minus BMD at baseline) at the femoral neck measurement site in the male intervention groups stratified by length of intervention. The number on top of the bars represents the number of participants in each subgroup. Error bars represent 1 SD

To our knowledge, there has only been a few other RCTs where the effect on BMD of vitamin D given alone has been studied. Thus, in the review and meta-analysis by Reid et al. in 2014 [29], 23 studies were identified where the interventions differed only in vitamin D content. However, vitamin D was given alone without calcium or other co-interventions in only seven studies, and among these, none but three included males. Of the studies including males, one included 50 subjects randomized to 300,000 IU vitamin D per year [30] and was excluded from the meta-analysis because of a 9-year age difference between the intervention groups; another included 173 subjects randomized to 400 IU, 800 IU, or placebo over 12 months where a non-significant positive effect at the lumbar spine and a significant negative effect at total BMD was found, however, not including measurements at the femoral neck [31]; and the third study was excluded due to not available nor obtainable quantitative data in the original publication [32]. As far as we are concerned, there has not been any studies with vitamin D alone that has included males published since 2014, and therefore it is fair to say that this has not been properly examined before.

In the present study, a positive effect of vitamin D supplementation was found only at the femoral neck measurement site. The femoral neck contains more cortical bone than what is included in the total hip measurement. The cortical bone is metabolically less active than the trabecular bone [33], and previous studies have shown that the cortical bone is also less responsive to treatment than the trabecular bone [34]. However, in the case of vitamin D deficiency, the secondary hyperparathyroidism causes bone loss mainly at cortical sites [35], and suppression of PTH, as was seen in our vitamin D group, could be the explanation for the BMD increase in the femoral neck. This was also found in the study by Ooms et al. [36] where vitamin D_3 400 IU/day versus placebo led to an increase of femoral neck BMD of 2% after 2 years, while there was no change at the trochanter. Moreover, these observations fit with the conclusion in the review by Reid et al. [29], in which a small, but significant effect was found at the femoral neck, but not at other measurement sites.

Yet, some limitations of our study ought to be considered. First, change in BMD was not the primary endpoint; thus, the study design may not have been appropriate. The inclusion criteria (IFG/IGT) favored subjects with high BMI, which is traditionally observed to have higher BMD due to mechanical loading and estrogen production via adipocyte aromatization [37]. Moreover, only a small number of subjects presented T-values corresponding with osteopenia, and accordingly, major improvements in BMD may therefore not have been likely. The influence of adipose tissue on bone metabolism is, however, not yet settled as recent studies indicate an inverse association between increased adiposity and low total BMD and total bone mineral content [38]. Additionally, studies have shown that T2DM patients are at higher risk of fracture when

Table 2 Predictors of baseline BMD in male and female subjects

Variables	Males				Females			
	Femoral neck (g/cm^2)	*p* value	Total hip (g/cm^2)	*p* value	Femoral neck (g/cm^2)	*p* value	Total hip (g/cm^2)	*p* value
Age (years)	− 0.180	0.003*	− 0.112	0.068	− 0.411	< 0.001*	− 0.328	< 0.001*
BMI (kg/m^2)	0.313	tbcolw30pt< 0.001*	0.334	tbcolw30pt< 0.001*	0.258	< 0.001*	0.336	< 0.001*
Tobacco use[e] (%)	− 0.128	0.029*	− 0.148	0.013*	− 0.151	0.035*	− 0.143	0.047*
tbcolw110ptVitamin D supplement use[a,b] (%)	0.025	0.668	− 0.021	0.715	− 0.005	0.943	− 0.047	0.519
Calcium supplement use[e] (%)	− 0.075	0.200	− 0.089	0.133	− 0.109	0.139	− 0.132	0.074
Serum 25(OH)D (nmol/L)	− 0.006	0.922	− 0.007	0.906	− 0.049	0.534	− 0.047	0.551
Serum calcium (mmol/L)	− 0.176	0.003*	− 0.128	0.032*	− 0.014	0.846	− 0.040	0.577
Serum PTH (pmol/L)	− 0.177	0.004*	− 0.127	0.041*	− 0.161	0.051	− 0.126	0.126
Serum creatinine (μmol/L)	0.171	0.005*	0.131	0.033*	0.119	0.121	0.059	0.445
HbA_{1c} (%)	0.006	0.921	0.059	0.313	0.101	0.165	0.097	0.185
R^2	0.238	< 0.001	0.216	< 0.001	0.330	< 0.001	0.323	< 0.001

Numbers represent standardized beta-coefficients and associated *p* values

[a] Including cod liver oil

[b] Coding: 0 = No, 1 = Yes

*Variable included in the linear regression model

they have incorrectly treated glucose levels [7]. Thus, an effect of vitamin D on fracture risk may have been shadowed in the present trial, as it was originally designed to remove all subjects developing T2DM.

Second, low serum 25(OH)D level was not an inclusion criteria at baseline, resulting in a wide range of serum 25(OH)D levels among the study participants. Baseline serum 25(OH)D levels were relatively high in the study population,

Table 3 Bone mass density measurements

		Vitamin D group	Placebo group	*p* value
Males (*n*)		125	131	
Femoral neck BMD (g/cm^2)	Baseline	0.974 ± 0.126	0.984 ± 0.136	0.561[a]
	Last visit	0.974 ± 0.124	0.973 ± 0.137	
	Delta	0.000 ± 0.029	− 0.010 ± 0.032	0.008[b,c]
Total hip BMD (g/cm^2)	Baseline	1.063 ± 0.137	1.078 ± 0.133	0.393[a]
	Last visit	1.065 ± 0.141	1.075 ± 0.14	
	Delta	0.002 ± 0.024	− 0.003 ± 0.03	0.149[a]
Females (*n*)		76	82	
Femoral neck BMD (g/cm^2)	Baseline	0.918 ± 0.117	0.887 ± 0.137	0.137[a]
	Last visit	0.900 ± 0.120	0.873 ± 0.143	
	Delta	− 0.017 ± 0.034	− 0.015 ± 0.029	0.592[a]
Total hip BMD (g/cm^2)	Baseline	1.003 ± 0.129	0.961 ± 0.140	0.055[a]
	Last visit	0.986 ± 0.130	0.943 ± 0.150	
	Delta	− 0.017 ± 0.034	− 0.018 ± 0.029	0.813[a]

Numbers represent mean ± SD

BMD bone mass density (g/cm^2), *Delta* $BMD_{Last\ visit} - BMD_{Baseline}$

[a] Student's *t* test

[b] Linear regression adjusting for baseline values, observation time and predictors of baseline BMD (Table 2)

[c] $R^2 = 0.082$

and thus, one might not expect major effects of further supplementation with vitamin D. Nevertheless, a small positive effect on BMD was observed in men. Moreover, subgroup analyses of data from subjects with baseline serum 25(OH)D levels below 50 nmol/L did not show significant effects; however, this might be explained by lack of statistical power, as the subgroup consisted of only a small number of subjects.

Third, length of intervention also varied among the study participants, with approximately 53% completing the 5-year trial. In short, median observation time was the same in the vitamin D group compared to the placebo group in males, while being longer in the female vitamin D group compared to placebo. However, BMD was found to increase in the male vitamin D group only, and when comparing median observation time between intervention groups, differences were non-significant for both men and women.

Fourth, the effect of vitamin D supplementation on BMD was not observed in both sexes. However, sexual dimorphism is not a new nor surprising finding when it comes to skeletal physiology and bone metabolism [39]. On average, men are taller, have larger amounts of muscle mass and lower body fat percentage, as well as having greater peak bone mineral content and peak trabecular bone volume [40–43]. The establishment of gender differences in the cortical and trabecular bone is found to be regulated by androgen and estrogen bioactivity, through a dual mode of action of testosterone on the cortical and trabecular bone via both the androgen receptor and estrogen receptor alpha [37]. If regulation of bone turnover in women operates through more complex mechanisms than in men, these mechanisms might override potential effects of vitamin D supplementation on the bone in women. However, information regarding history of use and/or current use of hormonal drugs was not available in the present study, and adjustments for these factors was therefore not made.

Finally, the proportion of variance of BMD explained (R^2) by the models in our analyses was rather small, and the clinical implications of our findings may be of modest importance. A small increase in BMD does not necessarily mean successful prevention of falls and/or fractures, as the reduction in bone strength is not only determined by BMD, but also by bone dimensions, microstructure, and material properties [37]. DEXA is a projectional (two-dimensional) technique, and thus, cannot truly differentiate between the cortical and trabecular bone. Therefore, such measures of bone health cannot assess the less apparent qualitative changes that may be present due to impaired glucose metabolism. Unfortunately, measurements with techniques allowing a three-dimensional assessment of bone structure and microarchitecture, such as peripheral quantitative computed tomography (pQCT) scanning, were not available in the present study.

However, the study has some strengths, as it is the largest, longest-running, published RCT with vitamin D as the only intervention, and both dosage and length of intervention ought to have been sufficient in order to detect an actual effect on BMD.

In conclusion, we have found a positive effect of vitamin D on BMD in males, but confirmatory studies are needed, preferably with change in BMD as the primary endpoint, and levels of 25(OH)D below 50 nmol/L as inclusion criterion. Additionally, evaluating bone properties with other techniques, such as high-resolution pQCT, may provide valuable insights.

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Compliance with ethical standards

Conflicts of interest None.

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