



Vitamin D, carotid intima–media thickness and bone structure in patients with type 2 diabetes

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Abstract

Despite aggressive treatment of cardiovascular disease (CVD) risk factors individuals with type 2 diabetes (T2D) still have increased risk of cardiovascular morbidity and mortality. The primary aim of this study was to examine the cross-sectional association between total (25-hydroxy vitamin D (25(OH)D)) and risk of CVD in patients with T2D. Secondary objective was to examine the association between 25(OH)D and bone health. A Danish cohort of patients with T2D participating in a randomised clinical trial were analysed. In total 415 patients (68% men, age 60 ± 9 years (mean \pm s.d.), duration of diabetes 12 ± 6 years), including 294 patients (71%) treated with insulin. Carotid intima–media thickness (IMT) and arterial stiffness (carotid artery distensibility coefficient (DC) and Young's elastic modulus (YEM)) were measured by ultrasound scan as indicators of CVD. Bone health was assessed by bone mineral density and trabecular bone score measured by dual energy X-ray absorptiometry. In this cohort, 214 patients (52%) were vitamin D deficient (25(OH)D < 50 nmol/l). Carotid IMT was 0.793 ± 0.137 mm, DC was 0.0030 ± 0.001 mmHg, YEM was 2354 ± 1038 mmHg and 13 (3%) of the patients were diagnosed with osteoporosis. A 25(OH)D level was not associated with carotid IMT or arterial stiffness ($P > 0.3$) or bone health ($P > 0.6$) after adjustment for CVD risk factors. In conclusion, 25(OH)D status was not associated with carotid IMT, arterial stiffness or bone health in this cohort of patients with T2D. To explore these associations and the association with other biomarkers further, multicentre studies with large numbers of patients are required.

Key Words

- ▶ carotid intima–media thickness
- ▶ 25-hydroxyvitamin D
- ▶ bone structure
- ▶ cardiovascular disease and type 2 diabetes

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Introduction

Despite aggressive treatment of cardiovascular disease (CVD) risk factors individuals with type 2 diabetes (T2D) still have an about twofold increased risk of cardiovascular morbidity and mortality compared to the general population (1, 2, 3).

Carotid intima–media thickness (IMT) measured by ultrasound has in several epidemiological studies been demonstrated to be associated with prevalent CVD and is considered an independent predictor of future CVD events like myocardial infarction (MI), stroke and death. Carotid IMT has therefore been suggested as a suitable surrogate marker for CVD in the general population (4, 5) and in patients with T2D (6). Carotid artery distensibility coefficient (DC) and Young's elastic modulus (YEM) are markers of arterial stiffness and has been shown to correlate well with overall cardiovascular outcome (7, 8). DC represents a global stiffness measurement of the entire arterial wall based on a single measurement site, whereas YEM can detect more subtle changes in the relative proportions of the layering of the arterial wall (9, 10).

A number of studies indicate a high prevalence of vitamin D deficiency in the general population and diabetic cohorts (11, 12). Several epidemiological studies have reported an association between vitamin D deficiency and risk of CVD including hypertension, hyperlipidemia, carotid IMT, arterial stiffness, MI and obesity (13, 14, 15). Few studies have examined the association between vitamin D status and risk of CVD in patients with T2D (16, 17), and one study has reported an association between low 25-hydroxy vitamin D (25(OH)D) and increased carotid IMT in patients with T2D (18). In contrast, sufficient levels of vitamin D has been linked to improved endothelial function, inhibition of foam cell formation and suppression of macrophage cholesterol uptake in patients with T2D (19, 20).

Vitamin D's effect on bone health (fractures and bone mineral density (BMD)) is well established in the general population (21), while data from epidemiological studies in persons with diabetes – being less conclusive – suggest that vitamin D deficiency predisposes to β cell dysfunction, insulin resistance and T2D (11). Patients with T2D have increased risk of hip (22, 23), spine and extremity fractures (24) despite normal or increased BMD (25).

We hypothesize, that low 25(OH)D is associated with risk of CVD and reduced bone health.

This cross-sectional study aims to investigate the complex relationship between 25(OH)D, risk of CVD (measured as carotid IMT, carotid artery DC and YEM)

and bone health (measured as BMD and trabecular bone score (TBS)) in a Danish cohort of patients with T2D participating in a randomised clinical trial (The Copenhagen Insulin and Metformin Therapy (CIMT) trial) (26).

Materials and methods

The CIMT trial is an investigator-initiated, multicentre, randomised controlled study with a 2×3 factorial design. The trial was initiated in May 2008 and completed in December 2012 at eight hospitals in the Region of Copenhagen, Denmark, in accordance with ICH–GCP regulations and the Helsinki Declaration (trial registration ClinicalTrials.gov number NCT00657943) and approved by the Ethical Committee. Patients were recruited from diabetes clinics and signed informed consent if they met the following inclusion criteria: age > 30 years, BMI between 25 and 40 kg/m², estimated glomerular filtration rate (eGFR) > 60 ml/min (calculated using the Modification of Diet in Renal Disease (MDRD) equation), HbA1c > 7.5% (58 mmol/mol) and treated with oral hypoglycaemic agents and/or insulin. Exclusion criteria were recent history of CVD, cancer, renal failure, liver disease or other chronic diseases as assessed by the investigator (26). CVD was defined as a previous history of MI, stroke, coronary or vascular surgery. Of the 464 patients screened for participation in the CIMT trial, 27 patients were not meeting the inclusion criteria, 11 patients declined to participate and 11 patients were not included due to other reasons. Thus, a total of 415 Danish patients with T2D were included and randomised, by central concealed randomization stratified for age (above 65 years), previous insulin treatment and treatment centre.

The results presented were obtained at the baseline investigation of the trial. Baseline measurements included clinical examinations, fasting blood and urine samples, carotid ultrasound scan and dual energy X-ray absorptiometry (DXA) scans. Serum total 25(OH)D (25-hydroxy vitamin D3 plus 25-hydroxy vitamin D2) was measured with an electro-chemiluminescence immunoassay (ECLIA) competitive assay using the Cobas e411, Roche equipment based on a one-step sandwich assay. This method was used as routine analyses in our hospital. All samples were measured in one batch using identical batch numbers. The coefficients of variation (CV) for 25(OH)D was 6.4%. Analytical sensitivity was of 10.0 nmol/l. According to the Institute of Medicine vitamin D deficiency is defined as a 25(OH)D < 50 nmol/l, where treatment is recommended (27).

Carotid IMT and arterial stiffness markers, DC and YEM, were measured by ultrasound scans of the carotid arteries and performed by the same two lab technicians. The CV as measured by the s.d. of the log-transformed values was 10% between sonographers and between days (28). After 10 min of rest in the supine position the scanning was performed using a GE Healthcare (Waukesha, WI, USA) logic 9 with a nine linear (8 MHz) or a 12 linear (12 MHz) probe, initially, a rough cross-sectional scanning was made to localize possible plaques or stenosis. Peripheral blood pressure was measured with a validated oscillometric device measuring on the brachial artery on the dominant arm after 10 min of rest and immediately after ultrasound examination. We did not have the possibility to measure the central blood pressure in this study. Thereafter, a longitudinal scanning was made of the common carotid artery with storage of a dynamic sequence of 4–6 s for the measurement of carotid IMT. For the border detection and calculations, we used specialized software (vascular tools 5, Medical Imaging Applications, Coralville, IA, USA). The region of interest was defined as a segment of the far wall in common carotid artery devoid of focal plaques and spanning 5–10 mm with a centre 10 mm proximal to the bulb. The mean carotid IMT was calculated as the average of the mean IMT of the left and right common carotid artery. Relative compliance (carotid artery DC (mmHg)) of the common carotid artery was automatically calculated as a measure of the change in vessel volume from systole to diastole calculated from the equation: $(\text{radius in systole}^2 - \text{radius in diastole}^2) / (\text{radius in diastole}^2 \times \text{pulse pressure})$ where high/increased values are beneficial for the hemodynamic system. YEM (mmHg) reflects the intima–media layers tendency to be deformed elastically where low/reduced values are beneficial for the hemodynamic system.

Lumbar spine of L1–L4, total femur and femoral neck BMD was measured by DXA using a Hologic Discovery A, series 82800-A (Hologic, Bedford, MA, USA) and Software 12.6.2. *T*-scores referring to the Hologic database young normal mean BMD were calculated for each anatomical site. Osteopenia was defined as *T*-score below -1 but above -2.5 , and osteoporosis was defined as any *T*-score ≤ -2.5 (29). Lumbar spine TBS was derived for each spine DXA examination via TBS Software (TBS iNsight Software, version 1.8; Med-Imaps, Pessac, France) installed on the Hologic machine. TBS was calculated as the mean value of the individual measurements for vertebrae L1–L4, based on gray-level analysis of DXA images. TBS calculation is performed over the same region of interest as the BMD

measurement. Any fractured and/or arthritis vertebrae were excluded from computation.

Information on physical activity was obtained by questionnaires.

Results

Data were tested for normal distribution by QQ-plots and histograms. Baseline characteristics were compared using Student's *t*-test for continuous variables and χ^2 -test for categorical variables. Correlation analyses (Pearson/Spearman's ρ) and multivariate linear regression analyses were performed in addition. The selection of covariates was based on two approaches. Relevant variables including age, sex, BMI, HbA1c, smoking, eGFR, systolic blood pressure and alendronate treatment, were significantly correlated to the dependent variables and selected. In addition, covariates reported in the literature were included (see 'Results' section). Data are presented as median (range) for 25(OH)D and mean (\pm s.d.) or *n* (%) as appropriate and divided according to deficient and sufficient status of serum 25(OH)D. A two-sided *P* value <0.05 was considered statistical significant. The statistical software SPSS (version 19, IBM, Chicago, IL, USA) was used.

Baseline characteristics are shown in Table 1. In this population, carotid IMT was 0.793 ± 0.137 mm, 43% of the patients had osteopenia and 3% had osteoporosis.

Vitamin D (25(OH)D)

The median concentration of 25(OH)D was 48 (7–176) nmol/l in the total group of patients with T2D (*n*=415) and 214 (52%) of the patients were 25(OH)D deficient (<50 nmol/l). No significant differences were found in 25(OH)D levels according to season (*P*=0.15, data not shown) or physical activity (*P*=0.149, data not shown).

Analyses in patients with and without vitamin D deficiency

The vitamin D deficient patients were younger, had shorter duration of T2D, higher BMI, higher HbA1c, higher eGFR, lower systolic blood pressure and lower carotid IMT compared to the patients with sufficient levels of 25(OH)D in unadjusted analyses (see Table 1). Dividing data according to 25(OH)D ≤ 37 nmol/l (as suggested in (18)) did not reveal any association in the overall results (data not shown).

Table 1 Baseline characteristics^a.

Variables	All (n=415)	25-hydroxy vitamin D (<50 nmol/l) (n=214)	25-hydroxy vitamin D (≥50 nmol/l) (n=201)	P value ^a
Sex (female), n (%)	132 (32)	60 (28)	72 (36)	0.089
Age (years)	60±9	58±9	62±8	<0.001
Insulin treatment, n (%)	294 (71)	238 (76)	56 (64)	0.135
T2D duration (years)	12±6	12±6	13±7	0.029
BMI (kg/m ²)	32.2±4.2	32.7±4.2	31.5±4.1	0.003
HbA1c (%) (mmol/mol)	8.6±1.1/70±12	8.7±1.1/72±12	8.3±1.1/68±10	0.001
eGFR (ml/min)	128±44	135±48	120±32	<0.001
Systolic blood pressure (mmHg)	139±15	137±15	142±16	0.004
Diastolic blood pressure (mmHg)	82±9	83±9	82±9	0.232
Statin treatment, n (%)	375 (90)	193 (90)	182 (91)	0.901
Total cholesterol (mmol/l)	4.2±0.9	4.2±1.0	4.1±0.9	0.697
Current smokers, n (%)	63 (15)	37 (17)	26 (13)	0.195
Vitamin D 25(OH)D (nmol/l)	48 (7–176)	31 (7–49)	68 (50–176)	<0.001
Vitamin D supplement, n (%)	76 (18)	26 (12)	50 (25)	0.001
Calcium supplement, n (%)	55 (13)	15 (7)	40 (20)	<0.001
Alendronate treatment, n (%)	8 (2)	4 (2)	4 (2)	0.929
Carotid IMT (mm)	0.793±0.137	0.771±0.133	0.816±0.139	0.001
DC (mmHg)	0.0030±0.001	0.0026±0.001	0.0025±0.001	0.236
YEM (mmHg)	2354±1037	2318±1057	2392±1016	0.488
Previous CVD, n (%)	80 (19)	65 (20)	15 (17)	0.611
Normal bone status, n (%)	218 (53)	111 (52)	107 (53)	0.964
Osteopenia, n (%)	177 (43)	92 (46)	85 (42)	–
Osteoporosis, n (%)	13 (3)	7 (3)	6 (3)	–
Total femur T-score	0.12±1.045	0.19±1.074	0.05±1.017	0.157
Total femur BMD (g/cm ²)	0.999±0.210	0.100±0.202	0.993±0.219	0.611
Femoral neck T-score	−0.59±1.0	−0.58±0.951	−0.60±1.051	0.885
Femoral neck BMD (g/cm ²)	0.811±0.175	0.811±0.165	0.811±0.186	0.986
Lumbar spine T-score	0.09±1.542	0.08±1.502	0.10±1.589	0.852
Lumbar spine BMD (g/cm ²)	1.067±0.227	1.074±0.198	1.058±0.254	0.475
TBS (unit less)	1.145±0.162	1.134±0.167	1.157±0.156	0.154

Carotid IMT, carotid intima–media thickness; DC, distensibility coefficient; YEM, Young's elastic modulus; CVD, cardiovascular disease; BMD, bone mineral density; TBS, trabecular bone score; eGFR, estimated glomerular filtration rate.

^aUnadjusted difference between patients with vitamin D deficiency and sufficiency. Values are shown as means (±s.d.), median (range) and n (%). Differences were assessed by Student's *t*-test (for continuous variables) and by the χ^2 -test (for categorical variables).

Analyses in patients with and without vitamin D supplementation

A total of 76 (18%) patients received vitamin D supplement and had significantly higher 25(OH)D (62 (12–176) nmol/l) compared to the patients not receiving vitamin D supplement (45 (7–161) nmol/l) ($P<0.001$). Patients receiving vitamin D supplement were older, had lower HbA1c, and higher cholesterol levels. A higher proportion of women received supplements (data not shown).

Vitamin D (25(OH)D) and CVD risk

25(OH)D was positively associated with carotid IMT ($P=0.002$), however after adjustment for sex, age, BMI, smoking, systolic blood pressure, total cholesterol, HbA1c and eGFR, the association was no longer significant ($P=0.36$) (see Table 2). Dividing the patients according

to vitamin D supplement did not reveal any association between 25(OH)D and carotid IMT (data not shown). Age and systolic blood pressure were significantly associated to carotid IMT, DC and YEM both in all patients and after exclusion of patients on vitamin D supplement in the multivariate regression analyses ($P<0.001$, data not shown). 25(OH)D was negatively associated with DC ($P=0.05$), but no significant association was found in the adjusted analyses ($P=0.61$) (see Table 2). No association was found between 25(OH)D and YEM (see Table 2).

Vitamin D (25(OH)D) and bone health

25(OH)D was not associated with total femur *T*-score, femoral neck *T*-score or lumbar spine *T*-score (see Table 2). Vitamin D status and TBS were positively associated ($P=0.02$). After adjustment for sex, age, BMI, smoking, calcium supplement, alendronate treatment and eGFR

Table 2 Multivariate linear regression analyses^a.

	25(OH)D (<i>n</i> =415)		
	<i>r</i>	<i>P</i> value	<i>P</i> value ^b (adjusted model)
Bone health			
Total femur <i>T</i> -score	−0.09	0.07	0.66
Femoral neck <i>T</i> -score	−0.03	0.56	0.68
Lumbar spine <i>T</i> -score	−0.04	0.37	0.59
TBS	0.115	0.02	0.94
CVD risk			
Carotid IMT	0.15	0.002	0.36
DC	−0.10	0.05	0.61
YEM	0.09	0.10	0.3

TBS, trabecular bone score; carotid IMT, carotid intima–media thickness; DC, distensibility coefficient; YEM, Young's elastic modulus.

^aDifferences between dependent and independent variables are tested with Spearman ρ analyses and multivariate linear regression analyses.

^bAnalyses are adjusted for sex, age, BMI, smoking, calcium supplement, alendronate treatment and eGFR in the analyses with bone health as dependent variables. Analyses of CVD are adjusted for sex, age, BMI, smoking, systolic blood pressure, total cholesterol, eGFR and HbA1c.

this association was no longer significant ($P=0.94$). Alendronate treatment and BMI were significantly positive associated with bone health, whereas smoking and bone health were negatively associated in the multiple regression analyses (data not shown).

No association was found between carotid IMT and any of the included measures of bone health.

Discussion

This study investigates the complex association between vitamin D, risk of CVD and bone health in a Danish cohort of obese patients with T2D. In contrast to previous studies in patients with or without T2D, no independent associations were found between vitamin D status and risk of CVD or bone health.

In the general population, two large cross-sectional studies have investigated the association between vitamin D status and risk of CVD, including measures of carotid IMT (13, 14), showing strong and independent positive relations between vitamin D deficiency (defined as 25(OH)D < 50 nmol/l) and subclinical as well as prevalent CVD, being persistent after adjustment for confounders. One study has also found a significant association between vitamin D levels and arterial stiffness (measured by pulse wave velocity) in a non-diabetic population (15). No studies have investigated the association between vitamin D status and the applied arterial stiffness markers, DC and YEM.

Similarly, several cross-sectional studies in the general population have found positive and significant

associations between vitamin D levels and BMD measures (30, 31). However, these associations were not found in the current study.

In patients with T2D, the association between vitamin D status and carotid IMT has only been analysed in one study previously. This case–control study of 390 cases (T2D) and 390 controls (normoglycaemic) concluded that vitamin D deficiency (defined as serum 25(OH)D ≤ 37 nmol/l) is more pronounced in patients with T2D compared to controls, and showed a strong and independent association with increased carotid IMT after adjustment for similar covariates as in our study in addition to use of medication (18). Dividing our data according to 25(OH)D ≤ 37 nmol/l did not reveal any differences in the results (data not shown). The main difference between the two studies is that patients in the present study are more obese, have lower vitamin D levels, thinner carotid IMT and more patients are on statin treatment (18). One study has investigated vitamin D levels and arterial stiffness (measured as pulse wave velocity) in 305 patients with T2D adjusted for similar covariates as in the current study, showing that low vitamin D status was associated with increased arterial stiffness (32). Our study suggests that in patients with T2D, the observed association between 25(OH)D and risk markers of CVD in the unadjusted analyses is primarily due to coexisting CVD risk factors, in particular age and systolic blood pressure.

Prospective cohort studies suggest that patients with T2D possess an overall higher risk of osteoporotic fractures compared to persons without T2D, despite normal BMD values (33). In patients with T1D predominantly normo-weight decreased BMD has consistently been observed (34). The effect of vitamin D on bone health in patients with T2D is sparsely investigated. One study found that male T2D patients with a vitamin D level < 50 nmol/l had an increased risk of vertebral fractures, whereas this was not the case in women ($n=161$), which could be due to a significantly higher proportion of men being smokers (35). In view of this study, it is notable that no significant association was found between vitamin D status and bone health in the present study. One possible explanation for this discrepancy could be the higher BMI in the present study since higher weight load seems beneficial for bone health, and patients included in the current study are markedly obese (BMI > 30 kg/m²).

The strengths of the current study are the size of the study cohort and the applied measurements. The study cohort consisted of a well-defined group of patients with T2D. All patients had their primary outcome measurements (ultrasound and DXA scans) performed in a

standardized manner in a single center to minimize variation. Since known CVD risk markers can only partly explain the increased risk of CVD in patients with T2D, assessing CVD risk markers by ultrasound scan is relevant although the method is currently only used for research purposes. Studies with longitudinal data of carotid IMT scans and clinical CVD outcomes in diabetic populations are needed. DXA scans are validated and considered the standard method to assess bone health, although BMD-derived measures from DXA scans may not always detect a potential increased fracture risk (36). For analyses of patients with T2D, this discrepancy seems to be more pronounced as these patients tend to have a different bone structure than the general population (33). In an attempt to improve data strength TBS analyses were included. However, although patients TBS levels was slightly degraded (according to manufacturer) no association was found to levels of 25(OH)D.

One limitation is that assessing vitamin D status can be associated with measurement uncertainty. The ECLIA applied in this study was re-standardised before the analyses. One study concluded that status of vitamin D measured by the ECLIA disagreed considerably with other assays, and that the assay generally overestimates the vitamin D concentration (37) implying that the prevalence of vitamin D deficiency is underestimated. Another study compared the ECLIA with RIA and showed acceptable limits of agreement (38).

Another limitation is that the current study did not include measurements of biomarkers like adipokines or inflammatory cytokines which might be involved. Further and larger studies are required to address the impact of biomarkers on the association between atherosclerosis and CVD risk.

Notably, studies including people with T2D will always have several confounders, because of distinct heterogeneity among participants in relation to age, BMI, physical activity, duration and severity of diabetes including prevalence of late complications. Furthermore, the CVD risk factors are very aggressively treated in patients with T2D.

After adjustment for relevant covariates, 25(OH)D was not associated with carotid IMT, arterial stiffness, or bone health in this cross-sectional study of patients with T2D.

Declaration of interest

LLC, TA, AV, OP, TB, BC, SL, TJ and LT hold shares in Novo Nordisk A/S; LLC, TA, AV, TB, BGR, SL and LT report former employment by Novo Nordisk; BC is employed at Steno Diabetes Center, which is a diabetes hospital and academic institution owned by Novo Nordisk; SL holds shares in

dynamically traded investment funds, which may own stocks from pharmaceutical companies; SL is an employee at Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany; SL's contribution was his alone and does not necessarily reflect the official position of Boehringer Ingelheim; AV has received fees from Novo Nordisk; TB is employed by Novo Nordisk A/S; BT is a member of an advisory board for Eli Lilly; OS has received fees from AstraZeneca, Sanofi, MSD, Boehringer-Ingelheim, Eli Lilly and Novo Nordisk; CH has received fees from Bristol-Myers Squibb, Sanofi and Novo Nordisk; LB has received fees from and attended advisory for Novo Nordisk A/S; SM has served as a consultant or adviser to Novartis Pharma, Novo Nordisk, Merck Sharp & Dome, Sanofi-Aventis, AstraZeneca, Johnson & Johnson, Rosche, Mankind, Astra-Zeneca, Boehringer-Ingelheim, Zealand, Eli Lilly, Intarcia Therapeutics and Bristol-Meyer Squibb; SM has received fees for speaking from Novo Nordisk, Merck, Sharp & Dome, Astra-Zeneca, Johnson and Johnson, Rosche, Shering-Plouf, Sanofi-Aventis, Novartis Pharma, Eli Lilly, Bristol-Meyer Squibb and Boehringer Ingelheim. SM has received 2 research grants from Novo Nordisk. PE is a board member at Eli Lilly, MSD and Amgen; PE is part of the speakers bureau with Amgen and Eli Lilly. KW, BH, JW, CG, NW, MR, HV, ED, HP, TK, SS and EM have no conflicts of interests to declare.

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