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# Association of total and bioactive serum sclerostin levels with bone metabolism in type 2 diabetes mellitus

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ABSTRACT

*Background:* Sclerostin has been associated with decreased bone turnover in patients with type 2 diabetes mellitus (T2DM). The relationship with bone turnover markers (BTMs) and bone mineral density (BMD) remains unclear. We investigate the relationship between total and bioactive sclerostin measured by three different assays with BTMs and BMD in patients with T2DM compared to healthy controls.

*Methods:* Baseline data from the cross-sectional multicenter DiabOS-study in Switzerland were analysed. Total and bioactive serum sclerostin levels were measured using three different ELISA-based sclerostin assays (Sclerostin Biomedica, Sclerostin bioactive Biomedica and Sclerostin hsTECO). Sclerostin levels in patients with T2DM and controls were correlated with BTMs and BMD.

*Results:* Data were analysed from 78 men and postmenopausal women with T2DM and 37 controls (aged 50–75 years). Serum sclerostin levels, adjusted for estimated glomerular filtration rate (eGFR), were higher in patients with T2DM compared to controls with all three assays. In a gender subgroup analysis, bioactive sclerostin levels remained significantly elevated in men with T2DM (T2DM, 106.8 ± 39.9 pmol/L; controls, 88.3 ± 21.3 pmol/L, $p = 0.03$).

Univariate analysis showed consistent significant correlations with all sclerostin assays for age, eGFR, glycated hemoglobin A1c and diabetes duration. However, in multivariate analysis, eGFR remained the only significant determinant of serum sclerostin levels. Sclerostin levels in patients with T2DM showed significant positive correlations with BMD but no significant correlations with BTMs.

*Conclusions:* We demonstrate a significant positive association of bioactive serum sclerostin with BMD at all measured sites in patients with T2DM, which may support its utility in the assessment of bone fragility in this population.

## Introduction

Longstanding and poorly controlled type 2 diabetes mellitus (T2DM) leads to microvascular and macrovascular complications and an increased risk of fragility fractures [1]. In a retrospective cohort study of patients with T2DM, the increased risk of osteoporosis appeared to be greater in patients younger than 65 years of age compared with controls without T2DM, highlighting the importance of reliable early detection of patients with T2DM with osteoporosis to ensure early disease control [2]. The underlaying mechanisms for the increased fracture risk are not fully understood. A complex interplay of various factors including structural abnormalities, increased vascular permeability due to inflammation as well as altered mesenchymal stem cell differentiation and altered gene expression in osteoblasts in a high glucose environment

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have been identified [3,4].

In a high glucose environment, an increased expression of sclerostin could be demonstrated in vitro and in animal studies [5,6]. In vivo, a pooled analysis demonstrated an increase in circulating sclerostin levels in patients with T2DM compared to healthy controls [7]. Bone homeostasis is largely dependent on the Wnt/beta-catenin signaling pathway: activation of this pathway results in mesenchymal cell differentiation into osteoblasts promoting bone formation. Sclerostin, which is primarily secreted by osteocytes, is a potent inhibitor of the Wnt/beta-catenin pathway and inhibits bone formation. Furthermore, sclerostin increases bone resorption by upregulation of RANKL (receptor activator of nuclear factor-kappa B ligand) and by downregulation of osteoprotegerin [8,9].

In a cross-sectional study analysing patients with T2DM and controls, Garcia-Martin et al. reported negative correlations of circulating sclerostin levels with bone turnover markers (BTMs) and positive correlations with bone mineral density (BMD) in patients with T2DM [10]. The Pearson's correlation coefficient for C-terminal cross-linked telopeptide of type I collagen (CTX) with serum sclerostin levels in patients with T2DM was $r = -0.363$ ($p = 0.002$). The age-adjusted Pearson's correlation coefficents in patients with T2DM for BMD at the lumbar spine, femoral neck and total hip with serum sclerostin levels were $r = 0.352$ ($p = 0.004$), $r = 0.511$ ($p < 0.001$) and $r = 0.521$($p < 0.001$), respectively. In addition, increased sclerostin levels were positively associated with diabetes duration ($\beta = 0.223$, $p = 0.064$) and with glycated haemoglobin A1c (HbA1c) ($\beta = 0.211$, $p = 0.074$) independently of age [10].

However, there are conflicting results depending on the type of immunoassay which was used to measure circulating sclerostin [11–13]. In addition, there is very limited data on correlation between sclerostin with BTMs and BMD in patients with T2DM. There is only scarce data on sclerostin levels in patients with T2DM, but higher sclerostin levels have been associated with increased vertebral fracture risk independently of BMD and BTMs [14,15]. However, with the available data the clinical interpretation of circulating sclerostin levels and its impact on bone metabolism in T2DM remains challenging.

There are several controllable and uncontrollable biological factors that influence circulating sclerostin levels. Circadian rhythm, physical activity and seasonal variation as well as gender, age and glucocorticoid use all affect circulating sclerostin levels [16]. Following the identification of the SOST gene, which encodes sclerostin [17], various methods have been used to measure sclerostin levels, including immunohistochemistry and Western blot analysis [18]. Sclerostin measurement became more accessible with the development of ELISA-based assays. Since then, several commercially available ELISA-based assays have been developed by different companies with different reference ranges and target protein binding sites [16]. As a result, comparative studies with different sclerostin assays have shown that different sclerostin assays measure different sclerostin concentrations [19–22]. Moreover, the binding epitope is only known for a few commercially available assays [16]. One of these is the Bioactive Sclerostin ELISA from Biomedica Medizinprodukte GmbH, which detects the sclerostin molecule at the binding site where it interacts with the Wnt-coreceptor LPR5/6 complex. The difference to all previous ELISA-based sclerostin assays is that the sclerostin molecule is detected at the site of interaction with the receptor, hence the term bioactive sclerostin. However, there is no data which part of the sclerostin molecule is actually bioactive. In a healthy Austrian population, circulating bioactive sclerostin measured with Bioactive Sclerostin ELISA was higher in men as compared to women and a positive correlation was observed with age, body mass index (BMI) and T-score at the total hip [23].

Furthermore, in an observational study, circulating bioactive sclerostin levels in Chinese postmenopausal women were negatively correlated with BTMs and positively correlated with BMD. There was no significant difference in bioactive sclerostin levels in patients with or without vertebral fractures [24]. Several biochemical markers of bone fragility have been studied in patients with T2DM, but so far only HbA1c has been reported to have additional value in fracture risk stratification [25].

The recent congruent associations between circulating bioactive sclerostin and bone metabolism in healthy populations in the aforementioned studies showed an advantage in sclerostin interpretation compared to the determination of total sclerostin with different sclerostin assays, which in the past had conflicting associations with BTMs and BMD, mainly due to the different known and especially unknown epitope recognition of the different immunoassays [11].

The aim of the present study was to analyse circulating serum sclerostin levels in a cohort of patients with T2DM and controls without T2DM and to investigate associations with BTMs and BMD. To this end, we compared serum sclerostin measured by three different assays, two commonly used assays for total sclerostin and the assay for measuring bioactive sclerostin.

## Materials and methods

### *Study population and design*

Baseline data were obtained from the DiabOS-study, a prospective multicenter observational cohort study evaluating skeletal health in patients with T2DM and controls without T2DM. Participants without fragility fractures with a complete baseline assessment were analysed for our study [26]. For DiabOS, men and postmenopausal women, aged from 50-75 years, were recruited at the University Hospital of Basel and the Cantonal Hospital of Lucerne, Switzerland, and via press advertisement. Patients with T2DM were enrolled if they had a documented diagnosis of T2DM for at least 3 years and if they were treated with oral antidiabetics or insulin. As this study was conducted as part of the DiabOS-study, the following methods were used as described in the study by Vavanikunnel et al, which evaluated the role of parathyroid hormone as a determinant of low bone turnover [26].

Exclusion criteria comprised any medical or psychiatric condition which would preclude the participants from adhering to the protocol, idiopathic or premenopausal osteoporosis, previous treatment with osteoporosis medication or intake of drugs known to affect bone metabolism (e.g. steroids, thiazolidinediones) within 6 months prior to enrolment or medical conditions known to affect bone health (e.g. metabolic bone disease such as primary hyperparathyroidism or Paget's disease, metastatic bone disease, thyrotoxicosis, hypercortisolism) [26].

During a standardised interview, data on detailed medical history with focus on T2DM and bone health, its treatment and the presence of diabetic complications were obtained and supplemented by medical records when appropriate. Fracture risk was assessed by fracture risk assessment tool (FRAX) launched by the University of Sheffield in 2008.

This cross-sectional study analysed baseline data from 115 participants, including 78 patients with T2DM and 37 controls without T2DM, after excluding 20 patients (7 patients with T2DM and 13 controls without T2DM) with a history of fragility fractures.

### *Biochemical assessment*

Fasting blood samples were taken in the morning (between 0800 h and 1100 h) and stored at −80 °C until analysis. Samples were analysed for HbA1c (Alere, Afinion), fasting glucose and insulin with the automated Elecsys® Insulin assay on a cobas e 411 analyzer (Roche Diagnostics International, Rotkreuz, Switzerland). Homeostatic model assessment estimates for insulin resistance (HOMA-IR) indicating the degree of insulin resistance was calculated based on insulin and glucose measurements (HOMA-IR = fasting insulin [uU/mL] * fasting glucose [mmol/L]/22.5).

Creatinine was analysed by standard method on an autoanalyzer (Hitachi System 704 analyzer; Roche Diagnostics International, Rotkreuz, Switzerland). C-terminal cross-linked telopeptide of type I collagen

(CTX), N-terminal propeptide of type I procollagen (P1NP), 25-Hydroxyvitamin D (25OH vitamin D, Vitamin D total) and intact parathyroid hormone (iPTH) were measured in serum with Elecsys® assays on the automated analyzer cobas e 411 (Roche Diagnostics International, Rotkreuz, Switzerland). The intra- and interassay variations were 2.0 and 8.4 % for CTX, 1.2 and 3.3 % for PINP, 2.2 and 10.7 % for 25OH vitamin D, and 1.2 and 2.0 % for iPTH, respectively [26]. Serum osteocalcin (OC) was measured with a N-MID osteocalcin assay on the automated IDS-iSYSY System (IDS, Bolton, UK). The intra- and interassay variations were <5 % and <10 %.

*Biochemical assessment of serum sclerostin*

Serum sclerostin was determined in duplicate using three different commercially available enzyme immunoassays: 1. Sclerostin ELISA (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria, Cat. No. BI-20492; SCL-BM), is a sandwich ELISA using a polyclonal goat anti-human sclerostin antibody as capture antibody and a monoclonal mouse anti-sclerostin-antibody as detector antibody. The epitope is not defined for this assay. However, it detects the whole molecule as well as fragments of the sclerostin molecule and therefore the bioactive form might also be detected to a certain degree as long as the LRP5/6 complex is present at the detected sclerostin molecule. According to the manufacturer, the standard range is from 0 to 240 pmol/L; 2. TECO® Sclerostin High sensitive (TECOmedical Group, Sissach, Switzerland, Cat. No. TE1023-HS; SCL-hsTECO), is a direct-capture immunoassay for quantification of sclerostin utilizing a polyclonal goat anti-human sclerostin antibody on a microwell plate and a monoclonal anti-human sclerostin antibody as secondary antibody. The epitope is not defined for this current assay. However, an earlier version of this assay detected the amino terminus and the mid-region of the sclerostin molecule. As long as the LRP5/6 complex is present at the detected molecule, this assay also might detects the bioactive sclerostin molecule to a certain degree. According to the manufacturer, the standard range is from 0 to 2 ng/mL; 3. Bioactive Sclerostin (Biomedica Medizinprodukte GmbH & Co KG, Vienna, Austria, Cat. No. BI-20472; SCL-bioBM) is a sandwich enzyme immunoassay composed of a recombinant human monoclonal sclerostin antibody as primary antibody and a conjugated polyclonal goat anti human sclerostin antibody as secondary antibody. The precoated monoclonal capture antibody detects sclerostin at an epitope located in the 2nd loop of the sclerostin molecule, the binding site of the low-density lipoprotein receptor-related protein 5/6 (LRP5/6). The epitope for this ELISA-based assay is known as described above. Therefore, the bioactive sclerostin molecule is specifically detected over all other commercially available ELISA-based assays. According to the manufacturer, the standard range is from 0 to 320 pmol/L.

Biomedica's ELISA-based assays are expressed in pmol/L. This unit indicates the number of molecules (sclerostin) in picomoles (1 pmol = $10^{-12}$ mol) per litre. The ELISA-based assay by TECOmedical Group is expressed in ng/mL. This unit indicates the mass of a substance (sclerostin) in nanograms (1 ng = $10^{-9}$ g) per millilitre. The molar mass of the sclerostin molecule is 22.5 kDa, which allows the measured sclerostin values to be converted into both units (1 pg/mL = 0.044 pmol/L), according to the manufacturer.

*Assessment of bone mineral density*

BMD at the lumbar spine, hip, proximal femur, and distal radius were measured by dual X-ray absorptiometry (DXA) using a Hologic Discovery densitometer (Hologic, Bedford MA, USA). To assess the short-term precision of the system in this population, a single repeat measurement in 20 patients was performed [27]. The coefficient of variation of individual measurements in our laboratory was 1.1 % for the spine, 1.4 % for the femoral neck, and 1.1 % for total hip [26].

Trabecular bone score (TBS) measurements at lumbar spine were performed using the TBS iNsight Software (version 1.8; Med-Imaps, Pessac, France).

*Statistical analysis*

Descriptive statistics of categorical parameters were summarized as counts and percentages. Chi-square test or the Fisher's exact test were used to compare patients with T2DM and controls without T2DM. Continuous parameters were expressed as means and standard deviations (SD). Mann-Whitney *U* test was used to compare the two groups. The given means of biochemical markers of bone metabolism and bone morphological values were adjusted for age and BMI in men and for age, BMI and hormone replacement therapy (HRT) in women.

Pearson's correlations coefficients and univariate linear regression analysis were calculated between each of two of the three sclerostin assays.

Pearson's correlation coefficients were calculated between sclerostin and the factors age, BMI, HbA1c, diabetes duration, estimated glomerular filtration rate (eGFR), P1NP, CTX, OC and BMD of lumbar spine, hip, proximal femur, and distal radius. Linear regression models were used to calculate the univariate and multivariate association between sclerostin with different factors or characteristics.

The level for statistical significance was set to an alpha $< 0.05$.

The software Statistical Analysis System 9.4 (SAS Institute, Cary NC, USA) was used for the analysis.

*Registration, ethics and informed consent*

As we performed a data analysis within the DiabOS-cohort as part of the registered DiabOS-study (NCT02551315) approved by the local ethics committee (Ethikkommission Nordwest- und Zentralschweiz, EKNZ 2015-117), no additional ethical application was required. The study was conducted according to the ethical guidelines of the Declaration of Helsinki, the International Conference on Harmonization guidelines on Good Clinical Practice (GCP) and national legal and regulatory requirements. Patient data was managed in accordance with GCP using secuTrial®. Written informed consent was obtained from all study participants.

## Results

*Baseline characteristics*

We included 115 participants with complete baseline data set without fragility fractures (Table 1). Patients with T2DM (n = 78) had a mean duration of diabetes of 13.4 ± 7 years with a mean HbA1c of 7.7 ± 1.3 % at the time of enrolment. 46 out of 78 patients with T2DM (40 %) were on insulin therapy with a mean treatment duration of 6.7 years (daily insulin dosages, 8 to 180 IU). Baseline characteristics showed significant higher BMI in patients with T2DM. 43 participants with T2DM and eight controls met the criteria for obesity (BMI ≥ 30 kg/m$^2$). Estimated renal function was equal in both groups, irrespective of gender. 13 study participants had a chronic renal insufficiency KDIGO Grade 3A or 3B (T2DM, n = 13; controls, n = 0) with a minimal eGFR of 34 mL/min. Macro- and microvascular complications were only observed in patients with T2DM with 10.3 % and 57.7 %, respectively. Postmenopausal hormone replacement therapy was prescribed significantly more often in controls (n = 16) compared to patients with T2DM (n = 3). There was no difference in calculated fracture risk for major osteoporotic fractures assessed by FRAX after adjustment for trabecular bone score.

*Biochemical markers of bone metabolism and bone characteristics*

Low bone turnover with significantly lower BTMs has been observed in patients with T2DM as compared to controls, independently of gender (Table 2). Both study groups had a sufficient mean 25-hydroxyvitamin D

**Table 1**
Baseline characteristics.

| Characteristics | All (n = 115) | | | Men (n = 71) | | | Women (n = 44) | | |
|---|---|---|---|---|---|---|---|---|---|
| | T2DM | Control | p value | T2DM | Control | p value | T2DM | Control | p value |
| Study group, n (%) | 78 (67.8) | 37 (32.2) | NA | 59 (83.1) | 12 (16.9) | NA | 19 (43.2) | 25 (56.8) | NA |
| Age (yrs) | 63.0 ± 6.4 | 61.8 ± 5.9 | 0.32 | 62.9 ± 6.3 | 63.0 ± 7.3 | 0.98 | 63.5 ± 6.9 | 61.2 ± 5.2 | 0.23 |
| BMI (kg/m$^2$) | 30.0 ± 4.1 | 25.8 ± 4.9 | < 0.01* | 29.9 ± 4.0 | 25.4 ± 2.9 | < 0.01* | 30.4 ± 4.2 | 26.0 ± 5.7 | 0.01* |
| eGFR (mL/min) | 85.3 ± 19.3 | 86.9 ± 11.6 | 0.73 | 86.3 ± 17.6 | 83.6 ± 14.3 | 0.48 | 81.9 ± 24.1 | 88.5 ± 10.0 | 0.68 |
| HbA1c (%) | 7.7 ± 1.3 | 5.5 ± 0.3 | < 0.01* | 7.8 ± 1.2 | 5.5 ± 0.3 | < 0.01* | 7.2 ± 1.4 | 5.6 ± 0.3 | < 0.01* |
| Diabetes duration (yrs) | 13.4 ± 7.0 | NA | NA | 13.2 ± 7.4 | NA | NA | 13.8 ± 5.8 | NA | NA |
| HOMA-IR | 6.5 ± 6.8 | 2.0 ± 1.2 | < 0.01* | 6.8 ± 7.1 | 1.7 ± 0.5 | < 0.01* | 5.3 ± 5.6 | 2.2 ± 1.4 | 0.01* |
| Insulin use, n (%) | 46 (40) | NA | NA | 40 (56.3) | NA | NA | 6 (13.6) | NA | NA |
| FRAX MOF (%) | 7.2 ± 4.7 | 7.7 ± 4.0 | 0.39 | 6.7 ± 4.4 | 5.9 ± 1.9 | 0.83 | 9.0 ± 5.0 | 8.4 ± 4.4 | 0.52 |
| Parent hip fracture, n (%) | 7 (6.1) | 3 (2.6) | 0.88 | 6 (8.5) | 0 (0) | 0.25 | 1 (2.3) | 3 (6.8) | 0.44 |
| Current smoking, n (%) | 13 (11.3) | 7 (6.1) | 0.26 | 9 (12.7) | 2 (2.8) | 0.90 | 4 (9.1) | 5 (11.4) | 0.93 |

Data expressed in mean ± standard deviation or n (%). * Significant p value < 0.05 for comparison between two groups.
T2DM, type 2 diabetes mellitus; BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin A1c; HOMA-IR, homeostatic model assessment estimates for insulin resistance; FRAX, fracture risk assessment tool (adjustments made for trabecular bone score); MOF, major osteoporotic fracture.

**Table 2**
Biochemical markers of bone metabolism and bone characteristics.

| Biochemical markers and bone characteristics | Men (n = 71) | | | Women (n = 44) | | |
|---|---|---|---|---|---|---|
| | T2DM (n = 59) | Control (n = 12) | p value (absolute difference) | T2DM (n = 19) | Control (n = 25) | p value (absolute difference) |
| P1NP (ng/mL) | 35.1 ± 11.3 | 47.1 ± 8.5 | < 0.01* (12 ng/mL) | 35.7 ± 11.2 | 60.2 ± 27.3 | < 0.01* (24.5 ng/mL) |
| CTX (ng/mL) | 0.2 ± 0.1 | 0.4 ± 0.1 | < 0.01* (0.2 ng/mL) | 0.3 ± 0.1 | 0.5 ± 0.2 | < 0.01* (0.2 ng/mL) |
| Osteocalcin (ng/mL) | 10.3 ± 4.5 | 16.4 ± 5.2 | < 0.01* (6.1 ng/mL) | 11.4 ± 4.6 | 18.0 ± 7.7 | < 0.01* (6.6 ng/mL) |
| iPTH (ng/L) | 43.1 ± 17.9 | 47.0 ± 11.8 | 0.34 | 34.4 ± 12.1 | 47.9 ± 24.2 | 0.04* |
| 25(OH) D (nmol/L) | 54.1 ± 24.8 | 72.5 ± 32.8 | 0.10 | 59.6 ± 27.3 | 64.2 ± 20.8 | 0.73 |
| Lumbar spine BMD (g/cm$^2$) | 1.07 ± 0.15 | 0.99 ± 0.16 | 0.29 | 1.00 ± 0.12 | 0.88 ± 0.12 | < 0.01* |
| T-Score | −0.69 | −0.79 | | −0.72 | −0.74 | |
| Femoral neck BMD (g/cm$^2$) | 0.83 ± 0.12 | 0.76 ± 0.09 | 0.51 | 0.80 ± 0.10 | 0.75 ± 0.12 | 0.17 |
| T-Score | −0.94 | −0.97 | | −0.94 | −0.96 | |
| Total hip BMD (g/cm$^2$) | 1.03 ± 0.14 | 0.92 ± 0.07 | 0.14 | 0.97 ± 0.11 | 0.88 ± 0.12 | 0.05 |
| T-Score | −0.34 | −0.36 | | −0.36 | −0.39 | |
| Distal radius BMD (g/cm$^2$) | 0.65 ± 0.07 | 0.61 ± 0.09 | 0.13 | 0.57 ± 0.07 | 0.54 ± 0.07 | 0.07 |
| T-Score | −0.61 | −0.71 | | −0.58 | −0.66 | |
| Trabecular bone score | 1.24 ± 0.16 | 1.34 ± 0.10 | 0.82 | 1.22 ± 0.15 | 1.31 ± 0.13 | 0.84 |

Data expressed in mean ± standard deviation. * Significant p value < 0.05 (adjusted for BMI and age in men and for BMI, age and HRT in women) for comparison between two groups.
T2DM, type 2 diabetes mellitus; P1NP, propeptide of type I procollagen; CTX, c-terminal telopeptide; iPTH, intact parathyroid hormone; 25(OH) D, 25-hydroxyvitamin D; BMD, bone mineral density; BMI, body mass index; HRT, hormone replacement therapy.

level (above 50 nmol/L).

Bone mineral density at all different skeletal sites was numerically higher in patients with T2DM compared to controls, irrespective of gender (Table 2). The level of significance was only reached in women at the lumbar spine (T2DM, 1.00 ± 0.12 g/cm$^2$; controls, 0.88 ± 0.12 g/cm$^2$; p < 0.01). However, a non-significant lower TBS was measured in men and women with T2DM.

*Serum sclerostin levels measured with three different assays*

Serum sclerostin levels measured with all three sclerostin assays were significantly higher in patients with T2DM compared to controls without T2DM (Table 3). In subgroup analyses for gender, serum sclerostin levels remained significantly elevated in men with T2DM, only when measured with SCL-bioBM (T2DM, 106.8 ± 39.9 pmol/L; controls, 88.3 ± 21.3 pmol/L; p = 0.03). However, there was no difference in serum sclerostin levels measured with all three sclerostin assays in women with T2DM compared to controls.

*Correlation of sclerostin assays*

In our in-house laboratory, the mean coefficient of variation (CV) of the sclerostin value duplicates measured with SCL-BM, SCL-bioBM and SCL-hsTECO were 5.4 %, 3.2 % and 1.2 %, respectively. Overall, we saw a moderate, but significant correlations between all three different sclerostin assays with a Pearson correlation coefficient r ≥0.8 (Table 4).

**Table 3**
Serum sclerostin levels measured with three different ELISA-based assays.

| Sclerostin assay | All (n = 115) | | | Men (n = 71) | | | Women (n = 44) | | |
|---|---|---|---|---|---|---|---|---|---|
| | T2DM (n = 78) | Control (n = 37) | p value | T2DM (n = 59) | Control (n = 12) | p value | T2DM (n = 19) | Control (n = 25) | p value |
| SCL-BM (pmol/L) | 42.3 ± 18.4 | 33.8 ± 13.8 | 0.01* | 45.4 ± 18.4 | 38.9 ± 13.3 | 0.10 | 32.8 ± 15.0 | 31.4 ± 13.6 | 0.82 |
| SCL-bioBM (pmol/L) | 101.2 ± 38.4 | 83.1 ± 27.4 | 0.01* | 106.8 ± 39.9 | 88.3 ± 21.3 | 0.03* | 83.7 ± 27.2 | 80.7 ± 30.0 | 0.86 |
| SCL-hsTECO (ng/mL) | 1.1 ± 0.3 | 0.9 ± 0.3 | 0.02* | 1.1 ± 0.3 | 1.0 ± 0.4 | 0.30 | 1.0 ± 0.3 | 0.9 ± 0.2 | 0.40 |

Data expressed in mean ± standard deviation. * Significant p value < 0.05 (adjusted for eGFR) for comparison between two groups.
T2DM, type 2 diabetes mellitus; SCL-BM, Sclerostin ELISA BIOMEDICA; SCL-bioBM, Bioactive Sclerostin ELISA BIOMEDICA; SCL-hsTECO, Sclerostin TECO® High sensitive; eGFR, estimated glomerular filtration rate.

**Table 4**
Correlation analysis between each two of the three different ELISA-based sclerostin assays.

| Sclerostin assays | Pearson's r | p value |
|---|---|---|
| SCL-BM – SCL-bioBM | 0.87 | <0.01* |
| SCL-BM – SCL-hsTECO | 0.83 | <0.01* |
| SCL-bioBM – SCL-hsTECO | 0.80 | <0.01* |

* Significant p value < 0.05.
SCL-BM, Sclerostin ELISA BIOMEDICA; SCL-bioBM, Bioactive Sclerostin ELISA BIOMEDICA; SCL-hsTECO, Sclerostin TECO® High sensitive.

Univariate linear regression showed the strongest correlation between serum sclerostin levels measured by SCL-BM and SCL-bioBM ($R = 0.87$, $R^2 = 0.75$).

*Univariate and multivariate analysis*

Univariate analysis in all study participants for serum sclerostin levels measured with three different sclerostin assays showed consistent significant correlations for age, eGFR, HbA1c and diabetes duration. In a univariate linear regression model, we saw a strong negative correlation for eGFR and sclerostin. For SCL-BM, SCL-bioBM and SCL-hsTECO, R was 0.49, 0.53 and 0.66, respectively ($p < 0.01$).

In a multivariate analysis, eGFR remained the only significant determinant of serum sclerostin levels measured by all three sclerostin assays. Negative correlations in the multivariate linear regression model for eGFR and SCL-BM, SCL-bioBM and SCL-hsTECO were $R = 0.59$, $R = 0.75$ and $R = 0.79$, respectively ($p < 0.01$).

*Correlation analysis of bioactive sclerostin in patients with T2DM*

Serum sclerostin levels measured by SCL-bioBM in patients with T2DM showed significant positive correlations with BMD at all measured skeletal sites. Univariate linear regression analysis with significant positive relationships between SCL-bioBM and BMD ranged from $R = 0.26$ ($p = 0.02$) at femoral neck, $R = 0.36$ ($p < 0.01$) at distal radius, $R = 0.38$ ($p < 0.01$) at total hip to $R = 0.42$ ($p < 0.01$) at lumbar spine (Fig. 1). No significant correlation was found between bioactive serum sclerostin levels and TBS. However, there was no significant negative relationship between sclerostin and BTMs. No correlations were observed with HOMA-IR and BMI.

## Discussion

In this cross-sectional study, we observed that serum sclerostin levels were predominantly higher in male patients with T2DM compared to controls without T2DM. Sclerostin levels measured by three different ELISA-based sclerostin assays correlated well with each other and eGFR remained the only significant determinant of sclerostin levels in patients with T2DM. Furthermore, the bioactive sclerostin levels were positively associated with BMD and there was a trend for a negative association with BTMs.

*Sclerostin assay performance*

Our measured sclerostin levels were within the manufacturer's reference ranges for all three assays. Moreover, the CV of our sclerostin value duplicates measured with all three assays was below the

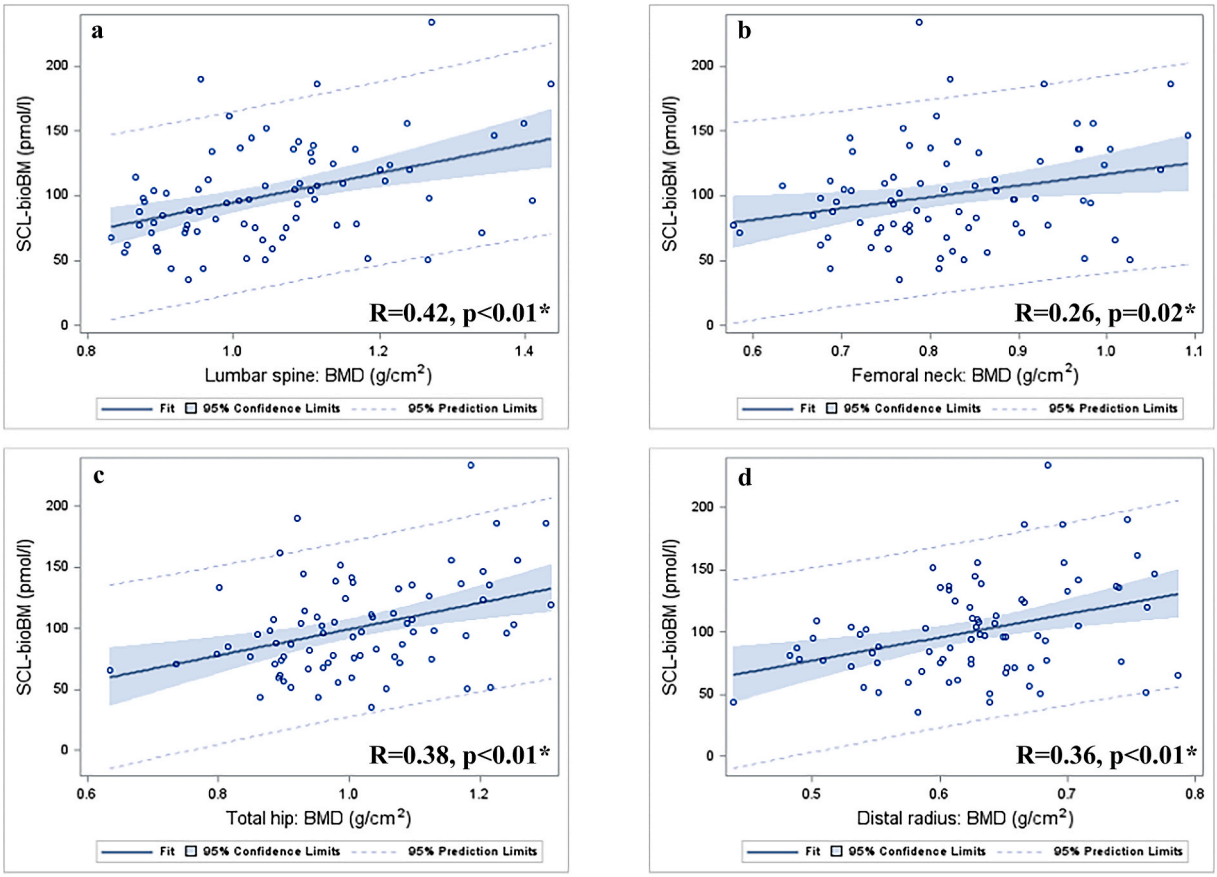


**Fig. 1.** Univariate linear regression analysis between bioactive serum sclerostin levels measured with Bioactive Sclerostin ELISA BIOMEDICA (SCL-bioBM) and bone mineral density (BMD) at lumbar spine (a), femoral neck (b), total hip (c) and distal radius (d) in patients with type 2 diabetes mellitus (n = 78). * Significant p value < 0.05. R, Pearson's correlation coefficient; p, p value for the linear association of BMD to SCL-bioBM.

manufacturer's CV (for SCL-BM $\leq$ 10 %, for SCL-bioBM $\leq$ 5 % and for SCL-hsTECO $\leq$ 5 %). Therefore, we consider the performance of all three assays to be sufficient. The correlation analysis between SCL-BM and SCL-bioBM showed a higher $R^2 = 0.75$ compared to the manufacturer's specifications of $R^2 = 0.58$. All assays used, showed a good correlation with each other ($R^2 \geq 0.64$). Although the assays of the different manufacturers were already comparable, the companies have continuously developed the assays further, which contributes significantly to the different results of the previous correlation analyses. [20,21,28].

The highest levels of sclerostin were measured with the SCL-bioBM assay, independent of gender and study group. A previous study demonstrated the detection of sclerostin fragments and a possible cross-reactivity with those fragments, resulting in higher sclerostin levels in assays from the Biomedica group such as the SCL-BM [29]. In addition, according to the Biomedica validation data report, the receptor binding site of the sclerostin molecule detected by SCL-bioBM is likely to be more robust to cleavage. As a result, higher levels of sclerostin are detected that more accurately reflect circulating sclerostin levels.

*Preanalytical difficulties*

The determination of serum sclerostin with today's data shows new potential in the assessment of bone metabolism, although especially preanalytical issues in particular are still not yet fully elucidated. For SCL-BM and SCL-hsTECO, the epitope binding site of the assay on the sclerostin molecule is still unknown. Knowing the exact binding site of the SCL-bioBM assay seems to be an advantage over the other assays, whereas the absolute sclerostin levels are still influenced by sample handling, controllable and uncontrollable biological variations [16].

Although the SCL-bioBM assay binds to the receptor interaction side of the sclerostin molecule, there is still no data which parts of the sclerostin molecule are biologically bioactive.

*Influence of gender and ethnicity*

Bioactive sclerostin values were reported to be higher in healthy men compared to healthy women [23]. Accordingly, we were able to demonstrate higher bioactive and total sclerostin levels in men, but also in patients with T2DM. The level of circulating sclerostin may be influenced by bone mass, as it was shown that the serum sclerostin levels are decreased in patients with osteoporosis compared to patients without osteoporosis in T2DM [10]. Especially in elderly people, the total-body bone mineral content could be a possible explanation for the gender difference [30]. Apart from bone mass as a possible factor for the sex differences in serum sclerostin levels measured by all three assays, we also see a reason in the small sample size in women due to lack of power. Ethnicity appears to have a major influence on sclerostin levels as well, which severely limits the generalization and comparability of correlations between sclerostin and bone metabolism [31].

*Influence of diabetes control*

Correlation analyses in our cohort with T2DM with SCL-bioBM showed moderate significant positive correlation with BMD and non-significant negative correlation with BTMs. These results correspond to the findings for BTMs [32] and also with measurements of the SCL-bioBM assay for BTMs and BMD [24] in healthy postmenopausal women. Our patients with T2DM were moderately controlled with a mean HbA1c 7.7 %. We assume not being able to report a significant association of diabetes duration and HbA1c in the multiple linear regression analysis as our cohort had a better diabetic control compared to previous data [10].

*Sclerostin excretion and different sclerostin compartments*

We were able to demonstrate a significant negative association with eGFR in the multiple linear regression analysis as the only determinant of sclerostin in our cohort. A significant negative correlation between eGFR and serum sclerostin was also shown by Register et al. in a previous study [13]. Interestingly, sclerostin excretion is inversely related to renal function. It was shown that sclerostin excretion increases as kidney function decreases [33]. Sclerostin levels in diabetic nephropathy may therefore be underestimated, complicating the interpretation in progressive diabetes complications. In more than half of our cohort with T2DM, we could observe macro- and microvascular complications. Increased local sclerostin production has also been observed in vascular calcification processes such as arteriosclerosis [34]. Although there may be extra-osseous sclerostin production in vascular calcification processes in our cohort, this cannot be distinguished from sclerostin from the bone compartment. There is also uncertainty about the clinical relevance of extraosseous local sclerostin production on bone metabolism. However, a few studies showed a moderate correlation of circulating sclerostin levels with different bone compartments, e.g. bone marrow [35] or sclerostin-positive osteocytic lacunae [36]. Whether this is transferable to the entire bone compartment with its cortical and trabecular impairments in T2DM is still unclear and not investigated.

*Limitations*

Apart from our well-balanced study population in both genders, there are several limitations regarding the interpretation of our findings. First, due to the cross-sectional study design, we are not able to report any causative relationships between sclerostin levels and bone metabolism. Second, because of a heterogeneity in distribution of patients with T2DM and controls, we adjusted the p values and performed gender-specific subgroup analysis to minimize this limitation. In addition, the non-significant results in the small female cohort most likely indicate a lack of power and limit the generalization of the results. In addition, our Swiss cohort was almost exclusively Caucasian (95.7 %), which limits generalisability to this population. Lastly, our study cohort has no history of fragility fractures. A statement about the potential benefit of sclerostin in fracture risk stratification and fracture risk prediction is not possible.

## Conclusion

We confirm significantly increased sclerostin levels in patients with T2DM, predominantly in men and particularly when measured with SCL-bioBM. According to clinical observation, SCL-bioBM performed best and seems to be a reliable alternative assay with consistent positive correlations of bioactive sclerostin levels with BMD at all measured sites. Despite the several reported determinants of sclerostin levels in previous studies, eGFR was the only significant determinant of sclerostin in our study population.

To establish routine measurement of circulating sclerostin in the future daily practice as an additional tool to evaluate bone metabolism in patients with T2DM, prospective studies with fracture endpoints with longitudinal fracture risk analysis as well as multi-ethnic validation are needed to assess the potential benefit in fracture risk stratification. However, there is a need for standardisation and clarification of the general analytical uncertainties in serum sclerostin measurement, whereby we interpret the measurement of the bioactive sclerostin levels as an important step towards minimising the analytical uncertainties.

## CRediT authorship contribution statement

**Cyril Traechslin:** Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Lilian Sewing:** Writing – review & editing, Resources, Data curation. **Sandra Baumann:** Writing – review & editing, Resources, Data curation. **Leticia Grize:** Writing – review & editing, Visualization, Formal analysis, Data

curation. **Janina Vavanikunnel:** Writing – review & editing, Resources, Data curation. **Marius Kraenzlin:** Writing – review & editing, Supervision, Resources. **Christoph Henzen:** Writing – review & editing, Supervision, Resources. **Christian Meier:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

[1] Compston J. Type 2 diabetes mellitus and bone. J Intern Med 2018;283(2):140–53. https://doi.org/10.1111/joim.12725.
[2] Lin HH, et al. Association between type 2 diabetes and osteoporosis risk: A representative cohort study in Taiwan. PLoS One 2021;16(7):e0254451. https://doi.org/10.1371/journal.pone.0254451.
[3] Picke AK, et al. Update on the impact of type 2 diabetes mellitus on bone metabolism and material properties. Endocr Connect 2019;8(3):R55–70. https://doi.org/10.1530/EC-18-0456.
[4] Miranda C, et al. Influence of high glucose and advanced glycation end-products (ages) levels in human osteoblast-like cells gene expression. BMC Musculoskelet Disord 2016;17(1):377. https://doi.org/10.1186/s12891-016-1228-z.
[5] Tanaka K, et al. Effects of high glucose and advanced glycation end products on the expressions of sclerostin and RANKL as well as apoptosis in osteocyte-like MLO-Y4-A2 cells. Biochem Biophys Res Commun 2015;461(2):193–9. https://doi.org/10.1016/j.bbrc.2015.02.091.
[6] Pacicca DM, et al. Elevated glucose acts directly on osteocytes to increase sclerostin expression in diabetes. Sci Rep 2019;9(1):17353. https://doi.org/10.1038/s41598-019-52224-3.
[7] Hygum K, et al. Mechanisms in endocrinology: Diabetes mellitus, a state of low bone turnover - a systematic review and meta-analysis. Eur J Endocrinol 2017;176(3):R137–57. https://doi.org/10.1530/EJE-16-0652.
[8] Delgado-Calle J, Sato AY, Bellido T. Role and mechanism of action of sclerostin in bone. Bone 2017;96:29–37. https://doi.org/10.1016/j.bone.2016.10.007.
[9] Dreyer TJ, et al. Novel insights on the effect of sclerostin on bone and other organs. J Endocrinol 2023;257(2). https://doi.org/10.1530/JOE-22-0209.
[10] Garcia-Martin A, et al. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2012;97(1):234–41. https://doi.org/10.1210/jc.2011-2186.
[11] Durosier C, et al. Association of circulating sclerostin with bone mineral mass, microstructure, and turnover biochemical markers in healthy elderly men and women. J Clin Endocrinol Metab 2013;98(9):3873–83. https://doi.org/10.1210/jc.2013-2113.
[12] Gennari L, et al. Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. J Clin Endocrinol Metab 2012;97(5):1737–44. https://doi.org/10.1210/jc.2011-2958.
[13] Register TC, et al. Sclerostin is positively associated with bone mineral density in men and women and negatively associated with carotid calcified atherosclerotic plaque in men from the African American-Diabetes Heart Study. J Clin Endocrinol Metab 2014;99(1):315–21. https://doi.org/10.1210/jc.2013-3168.
[14] Yamamoto M, Yamauchi M, Sugimoto T. Elevated sclerostin levels are associated with vertebral fractures in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2013;98(10):4030–7. https://doi.org/10.1210/jc.2013-2143.
[15] Ardawi MS, et al. High serum sclerostin predicts the occurrence of osteoporotic fractures in postmenopausal women: the Center of Excellence for Osteoporosis Research Study. J Bone Miner Res 2012;27(12):2592–602. https://doi.org/10.1002/jbmr.1718.
[16] Dincel AS, Jorgensen NR, I.-I.J.C.o.B.. Metabolism, new emerging biomarkers for bone disease: sclerostin and Dickkopf-1 (DKK1). Calcif Tissue Int 2023;112(2):243–57. https://doi.org/10.1007/s00223-022-01020-9.
[17] Balemans W, et al. Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet 2001;10(5):537–44. https://doi.org/10.1093/hmg/10.5.537.
[18] Bellido T, et al. Chronic elevation of parathyroid hormone in mice reduces expression of sclerostin by osteocytes: a novel mechanism for hormonal control of osteoblastogenesis. Endocrinology 2005;146(11):4577–83. https://doi.org/10.1210/en.2005-0239.
[19] Costa AG, Cremers S, Bilezikian JP. Sclerostin measurement in human disease: Validity and current limitations. Bone 2017;96:24–8. https://doi.org/10.1016/j.bone.2016.10.012.
[20] Piec I, et al. How accurate is your sclerostin measurement? comparison between three commercially available sclerostin ELISA Kits. Calcif Tissue Int 2016;98(6):546–55. https://doi.org/10.1007/s00223-015-0105-3.
[21] McNulty M, et al. Determination of serum and plasma sclerostin concentrations by enzyme-linked immunoassays. J Clin Endocrinol Metab 2011;96(7):E1159–62. https://doi.org/10.1210/jc.2011-0254.
[22] Clarke BL, Drake MT. Clinical utility of serum sclerostin measurements. Bonekey Rep 2013;2:361. https://doi.org/10.1038/bonekey.2013.95.
[23] Kerschan-Schindl K, et al. Circulating bioactive sclerostin levels in an Austrian population-based cohort. Wien Klin Wochenschr 2022;134(1–2):39–44. https://doi.org/10.1007/s00508-021-01815-0.
[24] Liang H, et al. Relationships between sclerostin and morphometric vertebral fractures, bone mineral density, and bone microarchitecture in postmenopausal women. Arch Osteoporos 2023;18(1):57. https://doi.org/10.1007/s11657-023-01235-9.
[25] Meier C, et al. Biochemical markers of bone fragility in patients with diabetes. J Clin Endocrinol Metab 2023;108(10):e923–36. https://doi.org/10.1210/clinem/dgad255.
[26] Vavanikunnel J, et al. Determinants of low bone turnover in type 2 diabetes-the role of PTH. Calcif Tissue Int 2022;111(6):587–96. https://doi.org/10.1007/s00223-022-01022-7.
[27] Glüer CC, et al. Accurate assessment of precision errors: How to measure the reproducibility of bone densitometry techniques. Osteoporos Int 1995;5(4):262–70. https://doi.org/10.1007/BF01774016.
[28] Costa AG, et al. Comparison of two commercially available ELISAs for circulating sclerostin. Osteoporos Int 2014;25(5):1547–54. https://doi.org/10.1007/s00198-014-2635-3.
[29] van Lierop AH, et al. The role of sclerostin in the pathophysiology of sclerosing bone dysplasias. Clin Rev Bone Miner Metab 2011;10(2):108–16. https://doi.org/10.1007/s12018-011-9123-5.
[30] Modder UI, et al. Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res 2011;26(2):373–9. https://doi.org/10.1002/jbmr.217.
[31] Alramah T, et al. Evaluating the correlation of sclerostin levels with obesity and type 2 diabetes in a multiethnic population living in Kuwait. Front Endocrinol (Lausanne) 2024;15:1392675. https://doi.org/10.3389/fendo.2024.1392675.
[32] Garnero P, et al. Association of serum sclerostin with bone mineral density, bone turnover, steroid and parathyroid hormones, and fracture risk in postmenopausal women: the OFELY study. Osteoporos Int 2013;24(2):489–94. https://doi.org/10.1007/s00198-012-1978-x.
[33] Cejka D, et al. Renal elimination of sclerostin increases with declining kidney function. J Clin Endocrinol Metab 2014;99(1):248–55. https://doi.org/10.1210/jc.2013-2786.
[34] De Mare A, et al. Sclerostin as regulatory molecule in vascular media calcification and the bone-vascular axis. Toxins (Basel) 2019;11(7). https://doi.org/10.3390/toxins11070428.
[35] Drake MT, et al. Effects of parathyroid hormone treatment on circulating sclerostin levels in postmenopausal women. J Clin Endocrinol Metab 2010;95(11):5056–62. https://doi.org/10.1210/jc.2010-0720.
[36] Mare A, et al. Clinical inference of serum and bone sclerostin levels in patients with end-stage kidney disease. J Clin Med 2019;8(12). https://doi.org/10.3390/jcm8122027.