Serum sclerostin levels in type 2 diabetes mellitus patients: possible correlations with bone metabolism parameters and thrombocytosis

Manal Ali Abdel Khalek[a], Amal Mohamad El-Barbary[a], Alyaa Ahmed Elsherbeny[c], Emad Abdel Mohsen Abdel Hadi[b], Mona Gameel Balata[d], Manal Shawky Hussein[a], Rasha Ahmad Gaber[b], Sonya Ahmed El-Gaaly[c]

[a]Departments of Rheumatology and Rehabilitation and [b]Biochemistry, Tanta University, Tanta, [c]Departments of Internal Medicine and [d]Rheumatology and Rehabilitation, Ain Shams University, Cairo, Egypt

Correspondence to Amal Mohamad El-Barbary, MD, Department of Rheumatology and Rehabilitation, Tanta University, Tanta 31111, Egypt
Tel: +20 100 126 4626; fax: 0403407734
e-mail: ml_barbary@yahoo.com

Received 01 october 2014
Accepted 01 November 2014

Egyptian Journal of Obesity, Diabetes and Endocrinology
2015, 1:21–27

Introduction
Type 2 diabetes mellitus (T2DM) is a group of pandemic debilitating metabolic diseases featuring chronic hyperglycemia that results from defective insulin secretion and/or insulin actions. Dame and Sutor reported that diabetic patients are prone to thrombocytosis through a complex interplay of mechanisms. Therefore, the aim of our work is to evaluate serum sclerostin levels in patients with T2DM and to analyze the relationships among sclerostin, bone mineral density (BMD), bone metabolism, and thrombocytosis.

Objective
This study aimed to evaluate serum sclerostin in T2DM and its correlations with bone metabolism and thrombocytosis.

Patients and methods
Fifty male T2DM patients were enrolled; they were divided into two groups according to existing thrombocytosis. Forty age-matched men were included as controls. Clinical tests of physical mobility, fasting blood glucose, glycated hemoglobin, calcium, creatinine, parathormone (PTH), 25-hydroxyvitamin D, bone-specific alkaline phosphatase (BALP), serum carboxy-terminal cross-linked telopeptide of type I collagen (sCTX-I), serum sclerostin, and BMD were performed.

Results
There were insignificant increases in BMD in diabetic patients versus controls. There were significantly lower levels of PTH, BALP, and sCTX-I in the diabetes mellitus (DM) patient groups compared with the controls ($P < 0.001$). Serum sclerostin levels were significantly higher in DM patients than the controls, with insignificantly higher sclerostin levels in group II. Serum sclerostin was correlated positively with disease duration and correlated negatively with PTH, BALP, and sCTX-I ($P < 0.001$).

Conclusion
Sclerostin plays a role in the pathogenesis of bone changes in T2DM. The interplay between vitamin D, PTH, and blood glucose highlights the possibility of an existing endocrine axis. Finally, the role of osteocytes in regulating hematopoiesis and association with DM and osteoporosis should be investigated further.

Keywords:
bone mineral density, sclerostin, thrombocytosis, type 2 diabetes mellitus

Egyptian Journal of Obesity, Diabetes and Endocrinology 1:21–27

2356-8062

Introduction

Type 2 diabetes mellitus (T2DM) is a group of pandemic debilitating metabolic diseases featuring chronic hyperglycemia that results from defective insulin secretion and/or insulin actions [1].

In addition to neurovascular, ocular, and renal complications, osteopenia and osteoporosis (OP) are major debilitating problems in diabetes mellitus (DM) patients. OP and several other DM complications (e.g. visual impairment and gait imbalance) increase the risk of falls, fragility, and fractures [2]. Although several investigators have addressed the question of how DM induces bone changes, the exact underlying mechanism is still unclear [3].

Although common age-related conditions (i.e. a decrease in sex hormone or vitamin D levels) or risk factors (i.e. reduced physical activity) may explain at least in part the association between diabetes and OP, detrimental skeletal effects of glucose toxicity, and insulin resistance or deficiency, adipose-derived hormones, diabetic complications, and pharmacological treatment have also been postulated [4].

Sclerostin is a secreted Wnt antagonist produced almost exclusively by osteocytes that binds to the low-density

lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6), inhibiting the canonical Wnt/b-catenin signaling pathway and thus osteoblast activity [5]. It has been reported that the levels of sclerostin were found to be increased in patients with T2DM [6].

Osteocytes are the most abundant cell type in the bone, which were originally believed to be rather inert cells whose function was limited to maintaining bone matrix locally. Because osteoblasts have been implicated in the maintenance of hematopoietic stem cells and B-cells, it was suggested that signals generated by osteocytes contribute toward the regulation of hematopoiesis [7]. Dame and Sutor [8] reported that diabetic patients are prone to thrombocytosis through a complex interplay of mechanisms. Therefore, the aim of our work is to evaluate serum sclerostin levels in patients with T2DM and to analyze the relationships among sclerostin, bone mineral density (BMD), bone metabolism, and thrombocytosis.

Patients and methods

Study design

Fifty male patients with a diagnosis of diabetes according to the American Diabetes Association criteria [9] were enrolled in the current study from among patients attending the Physical Medicine, Rheumatology and Internal Medicine Departments of Tanta University Hospitals. Patients were divided into two groups (group I included 40 patients with T2DM without existing thrombocytosis and group II included 10 T2DM patients with existing thrombocytosis). All patients had normal renal function (as assessed by serum creatinine levels). In addition, 40 apparently healthy age-matched men were included as a control group. All the controls included had normal glucose homeostasis as assessed by fasting glucose levels and measurement of glycated hemoglobin (HbA1c).

Informed consent was obtained from all participants in the study. All patients gave their formal consent. The protocol was approved the Ethical Comittee of the Faculty of Medicine Tanta University.

Exclusion criteria

Patients with chronic diseases other than T2DM, conditions that affect bone metabolism such as rheumatoid arthritis, endocrinal disease, and malignancy were excluded. Patients receiving treatment with antiresorptive or anabolic compounds for OP in the previous 2 months or receiving drugs that affect bone metabolism such as calcium supplements, vitamin D preparations, selective estrogen receptor modulators, calcitonin, estrogen therapy, thiazides, glucocorticoids, or anticonvulsants were also excluded.

Subject and Methods

All participants underwent the following:

Clinical assessment

Full assessment of history was performed, BMI was calculated using the Quetelet formula (weight in kg divided by the height in m^2), history of falls and history of fracture was assessed, and clinical tests of physical mobility were performed including the Berg Balance Scale [10] and the Timed Up and Go Test [11].

Laboratory assessments

Biochemical assays were performed on fasting morning blood samples obtained concurrently with assessments of clinical parameters. Blood smears were examined to verify platelet counts thrombocytosis was defined as a platelet level of more than 450 000/μl, in compliance with the revised World Health Organization Diagnostic Criteria [12] (the normal range for healthy individuals is 150 000–450 000/μl). After an hour at room temperature, all samples were centrifuged, then sera were transferred to fresh tubes in aliquots and kept at −70°C until the day of analysis.

Fasting blood glucose, HbA1c, calcium, and creatinine were measured using automated standard laboratory techniques. Levels of 25-hydroxyvitamin D (25-OH-D) were measured by enzyme-linked immunosorbent assay (ELISA) (BioVendor, Asheville, North Carolina, USA). Parathormone (PTH) was measured using ELISA (Gen Way Biotech Inc., San Diego, California, USA), serum bone-specific alkaline phosphatase (BALP) level was measured using ELISA (solid phase ELISA; Biomarkers, Seattle, Washington, USA), serum carboxy-terminal cross-linked telopeptide of type I collagen (sCTX-I) was measured using the ELISA kit (Human Cross-linked Carboxy-terminal Telopeptide of Type I Collagen, MyBioSource, San Diego, USA), and serum sclerostin was measured using a commercial sandwich ELISA assay (Biomedica Gruppe, Vienna, Austria); the detection limit of sclerostin ELISA was 3.2 pmol/l.

Radiological assessment: bone mineral density measurement

BMD of the lumbar spine (anterior–posterior projection at L1–L4) and hip (total proximal femur) were measured using DXA [Hologic QDR Discovery (UMCG) or Hologic QDR Delphi (MCL); Waltman, MA, USA]; the results were expressed as g/cm^2. According to the WHO classification, normal bone density was defined as T score ≥−1.0, osteopenia as −2.5 < T score < −1.0, and OP as T score ≤−2.5 [13].

Statistical analysis

All data were analyzed using software (version 11; SPSS Inc., Chicago, Illinois, USA). Baseline characteristics are presented as mean ± SD for continuous variables and as frequency and percentage for discrete variables. Comparisons between groups were carried out using analysis of variance. The correlation between variables was examined using Pearson's correlation coefficient. Multiple linear regression analysis was used to determine the independent predictors of serum sclerostin levels. A *P* value less than 0.05 was considered statistically significant.

Results

Fifty T2DM male patients were enrolled in this study: 40 patients without thrombocytosis (group I) and 10 patients with thrombocytosis (group II). The mean age of DM patients was as follows: 53.4 ± 7.3 years in group I and 56.23 ± 5.3 years in group II. The mean age of the controls was 57.6 ± 6.3 years, with insignificant differences among the three groups. Table 1 shows the demographic, clinical, and radiological parameters of the study population.

Comparisons between the diabetic groups and the controls indicated that diabetic patients showed worse performances ($P < 0.0001$) on the Berg Balance Scale and Timed Up and Go Test.

BMD showed an insignificant increase in the two groups of diabetic patients versus the controls. In group I, only six (17.5%) out of 40 patients were osteopenic. In addition, in group II, one (10%) out of 10 patients was osteopenic. However, among the controls, seven (15%) participants were osteopenic.

In terms of laboratory findings, serum levels of calcium and 25-OH-D were found to be insignificantly lower in our patients than in the controls. The current study showed significantly lower levels of PTH, BALP, and sCTX-I in the patient groups compared with the controls, whereas there was no significant difference between groups I and II (Table 2).

Our results showed significantly increased sclerostin levels in the T2DM groups compared with the controls, with an insignificantly higher level in thrombocytosis diabetic patients (group II).

Table 1 Demographic, clinical, and radiological data of type 2 diabetes mellitus patients and controls

Parameters	Group 1 T2DM patients (*n* = 40)	Group 2 T2DM patients (*n* = 10)	Controls (*n* = 40)
Age (years)	53.4 ± 7.3	56.23 ± 5.3	57.6 ± 6.3
BMI (kg/m^2)	30.76 ± 7.86	31.02 ± 5.43	28.67 ± 5.77
Diabetes duration (years)	15.3 ± 5.5	14 ± 6.8	–
History of falls [*n* (%)]	5 (12.5)	1 (10)	3 (7.5)
History of fracture [*n* (%)]	2 (5)	–	1 (2.5)
BBS	51 ± 3*	51 ± 2*	55 ± 1
TUG (s)	8.46 ± 1.35*	8.5 ± 1.22*	6.22 ± 0.85
BMD (g/cm^2)			
Lumbar spine	1.123 ± 0.17	1.122 ± 0.18	1.088 ± 0.15
T score	−0.58 ± 1.49	−0.56 ± 1.48	−0.63 ± 1.45
Proximal femur	1.132 ± 0.142	1.112 ± 0.150	1.037 ± 0.125
T score	−0.71 ± 1.23	−0.70 ± 1.21	−0.97 ± 1.14

The values represent the mean ± SD; BBS, berg balance scale; BMD, bone mineral density measurement; T2DM, type 2 diabetes mellitus; TUG, Timed Up and Go Test; *$P < 0.001$ versus controls.

Table 2 Laboratory data in type 2 diabetes mellitus patients and controls

Parameters	Group 1 T2DM patients (*n* = 40)	Group 2 T2DM patients (*n* = 10)	Controls (*n* = 40)
FBG (mg/dl)	185.1 ± 53.5*	174.1 ± 58.6*	87.9 ± 11.4
HbA1c (%)	8.2 ± 1.9*	8.1 ± 1.9*	4.8 ± 0.5
Creatinine (mg/dl)	0.9 ± 0.19	0.93 ± 0.18	0.87 ± 0.17
Calcium (mg/dl)	9.53 ± 0.43	9.52 ± 0.46	9.73 ± 0.54
PTH (pg/ml)	36.8 ± 17.1*	38.8 ± 16.3*	50.2 ± 20.65
25-OH-D (ng/ml)	18.14 ± 9.11	17.98 ± 10.1	20.34 ± 8.66
BALP (U/l)	15.52 ± 4.53*	15.90 ± 5.23*	22.0 ± 8.7
sCTX-I (ng/ml)	0.46 ± 0.62*	0.43 ± 0.52*	0.78 ± 0.54
Sclerostin (pmol/l)	59.51 ± 35.52*	64.51 ± 35.58*	45.51 ± 30.24

The values represent the mean ± SD; BALP, serum bone-specific alkaline phosphatase; FBG, fasting blood glucose; HbA1c, glycated hemoglobin; 25-OH-D, 25-hydroxyvitamin D; PTH, parathormone; sCTX-I, serum carboxy-terminal cross-linked telopeptide of type I collagen; T2DM, type 2 diabetes mellitus; *$P < 0.001$ versus controls.

In our study, sclerostin levels in T2DM showed a significant positive correlation with disease duration and HbA1c ($P < 0.001$). However, sclerostin levels were correlated negatively with PTH, BALP, and sCTX-I ($P < 0.001$) (Table 3).

Multiple linear regression of serum sclerostin levels showed that there were statistically significantly correlated independent factors, which were PTH ($\beta = -0.389$, $P < 0.001$), BALP ($\beta = -0.381$, $P < 0.001$), and sCTX-I ($\beta = -0.289$, $P = 0.009$).

Table 3 Correlations of sclerostin in type 2 diabetes mellitus patients and controls

Parameters	T2DM patients ($n = 50$)		Controls ($n = 40$)	
	r	*P*	*r*	*P*
Age (years)	0.123	NS	0.095	NS
Diabetes duration (years)	0.865	<0.001	0.324	NS
BBS	0.324	NS	0.235	NS
TUG (s)	0.235	NS	0.078	NS
BMD lumbar spine	0.078	NS	0.241	NS
BMD proximal femur	0.027	NS	0.224	NS
HbA1c (%)	0.783	<0.001	0.235	NS
PTH (pg/ml)	−0.964	<0.001	0.238	NS
25-OH-D (ng/ml)	0.127	NS	0.327	NS
BALP (U/l)	−0.675	<0.001	0.024	NS
sCTX-I (ng/ml)	−0.732	<0.001	0.135	NS
Platelets count	0.027	NS	0.224	NS

Abbreviations as in Tables 1 and 2

Discussion

OP and DM are two highly prevalent diseases that are associated with an increased risk of fragility fractures, and have a major impact on the morbidity and mortality of the general population. Although various observational studies have investigated the association between the two, the mechanism by which DM favors the appearance of fractures does not appear to have been established adequately [14]. However, it is widely accepted that hyperglycemia is a salient factor that exerts direct and indirect deleterious effects on osteoblast function and bone formation [3].

Our study showed an insignificant increase in BMD in diabetic patients versus controls and among the two patient groups. Chin *et al.* [15] and Thrailkill *et al.* [16] reported that T2DM patients show a modest increase in BMD than controls, whereas low BMD has consistently been observed in T1DM patients [17]. However, Tuominen *et al.* [18] and Maghbooli *et al.* [19] reported no clear association between BMD and T2DM. One of the hypotheses that explains increased BMD in T2DM is that insulin is an anabolic factor for bone. As a consequence, hyperinsulinemia in T2DM stimulates bone formation [20].

Paradoxically, a decrease in physical mobility and an increased risk of osteoporotic fracture in T2DM have been reported repeatedly and this was independent of BMD [21–23]. In addition, it has been well established that diabetic patients have impaired bone healing after fracture [24]. This led to the hypothesis that there are diabetes-associated alterations in material and structural properties. An overly glycated collagen matrix, confounded by a low turnover state, lipid accumulation in the marrow of long bones, leads to expansion of marrow cavity and thinning of the cortical envelope, leading to subtle cortical abnormalities, and the osteoblast-to-adipocyte shift might also reduce the number of differentiated osteoblasts available for bone formation. All these may lead to compromised biomechanical competence [25].

The current study showed that there were significantly lower levels of PTH, with insignificantly lower calcium and 25-OH-D levels in the patient groups compared with the controls, which is in agreement with the results of many studies [26–28]. Paula *et al.* [29] clearly showed that PTH secretion is impaired in patients with poorly controlled DM. Several mechanisms have been postulated to explain the low PTH levels, such as patients with DM usually have hypomagnesemia because of osmotic diuresis [30], a factor that is known to interfere with PTH synthesis. Also, studies with bovine parathyroid cell cultures have shown PTH inhibition with an excess of glucose or deficit of insulin [4]. Thus, it appears that both factors are independent and additive in inhibiting PTH. McNair *et al.* [31] have shown that serum calcium is slightly lower in DM than in controls. This can be attributed to increased calcium excretion in association with 'functional hypoparathyroidism' that is observed during poor blood glucose control and wastage of calcium in urine and also because of vitamin D insufficiency in these patients.

The reduced PTH in our patients can explain the increased levels of sclerostin as PTH plays an inhibitory role in sclerostin production in humans as described by several authors [17,32] and circulating sclerostin is reduced by intermittent PTH therapy [19].

The levels of BALP and sCTX-I were significantly lower in our patient groups compared with the controls, which is in agreement with Parkinson and Fazzalari [33], who reported that biochemical markers of bone formation have generally been shown to be reduced.

Recent reports propose that the Wnt signaling pathway may be implicated in the association between T2DM,

OP, and increased risk of fractures [2,34], and that its role may be crucial in the pathogenesis of impaired bone quality observed in these patients [14].

Sclerostin is one of the major regulators of the Wnt pathway that is expressed almost exclusively in osteocytes [16–17].

Our results showed significantly increased sclerostin levels in the two DM groups compared with the controls, with insignificantly higher levels in thrombocytosis diabetic patients.

This finding is in agreement with many authors who reported increased sclerostin levels in T2DM compared to controls [14,35–37]. This finding differs from previous data of sclerostin function derived from sclerosteosis [38] and Van Buchem's disease [39], given that the physiological role of sclerostin is the inhibition of osteoblast proliferation and activity, which would be expected to have a negative relationship with bone mass, thus highlighting the assumption of impaired Wnt signaling pathway in T2DM.

A possible hypothesis explaining this contradictory finding is that the increase in sclerostin levels means a decrease in bone formation on the basis of its physiological functions, and therefore leads to a decrease in bone turnover. A lower bone turnover would mean slowed bone loss and higher bone mass. Conceptually, high sclerostin serum levels would be indicative of decreased formation of bone markers [40].

Van Lierop *et al.* [36] clarified that men with T2DM have higher serum sclerostin levels than controls, and these levels further increase after treatment with pioglitazone, which is also associated with increased sCTX-I. These findings suggest that increased sclerostin production may be involved in the pathogenesis of increased skeletal fragility in patients with T2DM in general and may specifically contribute toward the detrimental effect of thiazolidinediones on bone.

In our study, sclerostin showed a positive correlation with disease duration and HbA1c, which is consistent with many authors. Meanwhile, sclerostin showed an inverse relationship with bone formation and resorption markers, indicating a state of slow turnover [34,40,41]. In theory, elevated concentrations of sclerostin ought to be associated with a decrease in markers for bone formation. Meanwhile, our results are not in agreement with other studies reporting no relationship between sclerostin and markers for bone remodeling [32,42].

We could not establish a correlation between sclerostin and BMD in T2DM; however, we did not find a significant relationship between serum sclerostin and biochemical markers of bone turnover or BMD in our controls.

Sclerostin levels were associated negatively with PTH in our patients. This finding is consistent with phosphocalcic balance alterations described in T2DM [42,43] and could explain in part the increase in sclerostin that we observed in T2DM. The reduced effect of PTH on bone might decrease inhibition of the *SOST* gene, which would stimulate the expression of sclerostin, a contrasting situation to that described in patients with primary hyperparathyroidism.

However, Andress *et al.* [44] reported slightly higher PTH levels in T2DM than in controls and this, under normal circumstances, might have led to reduced rather than increased sclerostin levels.

In our study, we attempted to establish a relationship between DM, bone metabolism, and thrombocytosis. This idea emerged from the finding that diabetic patients are prone to thrombocytosis [8] and that osteocytes contribute toward the regulation of hematopoiesis [45] and that other cell types, such as endothelial progenitor cells lining the blood vessels, are also affected by hyperglycemia [25].

We could recruit ten T2DM patients with thrombocytosis selected from among hundreds of patients, but we failed to find significant differences between those patients and others without thrombocytosis. They also showed the same relations as the nonthrombocytopenic group. This could be attributed to the small sample size or to the fact that thrombocytosis may not be exerting additional changes on bone metabolism. Meanwhile, the only reference found was the case report of Vani and Mohen [46] of one diabetic patient with thrombocytosis.

To test the hypothesis that signals generated by osteocytes contribute toward the regulation of hematopoiesis, Fulzele *et al.* [45] generated mice with a conditional deletion of GαS in osteocytes. GαS is an obligate subunit of several G protein-coupled receptors that are expressed in osteocytes. Surprisingly, the deletion of GαS in osteocytes resulted in a myeloproliferative-like syndrome characterized by neutrophilia, thrombocytosis, and splenomegaly. Indeed, deletion of GαS in osteocytes results in osteocyte expansion, loss of osteoblasts, and osteopenia. The authors show that increased osteocyte expression of sclerostin is responsible for the loss of osteoblasts. The study led to the discovery that osteocytes produce large amounts of granulocyte colony-stimulating factor, the principal cytokine regulating granulopoiesis. Meanwhile, they clarified that osteocytes play an

essential role in sensing and responding to mechanical stress on bone. Thus, osteocytes may provide a mechanism by which mechanical and other stresses on bone regulate hematopoiesis.

Conclusion

Bone fragility in T2DM does not appear to be reflected by BMD, which eliminates the possibility of using BMD as a marker for the risk of fracture in T2DM. Sclerostin seems to play a key role in the pathogenesis of bone changes in T2DM, with contradictory findings of increased sclerostin and reduced bone turnover markers and lack of association with BMD suggestive of impaired Wnt signaling pathway in T2DM. The interplay between vitamin D, PTH, and blood glucose highlights the possibility of an existing endocrine axis involving bone and glucose metabolism. This raises a question of whether it may be possible to eventually formulate a single therapy that can concurrently target OP and T2DM.

Finally, the assumed role of osteocytes in regulating hematopoiesis and their association with DM and OP should be investigated further to be clarified, and also to postulate whether selerostin-neutralizing antibodies can possibly be used in the future to correct thrombocytosis or leukocytosis.

Acknowledgements

Conflicts of interest

None declared.

References

1 American diabetes association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2009; 32:S62–S67.

2 Wongdee K, Charoenphandhu N. Osteoporosis in diabetes mellitus: possible cellular and molecular mechanisms. World J Diabetes 2011; 2:41–48.

3 Wang W, Zhang X, Zheng J, Yang J. High glucose stimulates adipogenic and inhibits osteogenic differentiation in MG-63 cells through cAMP/protein kinase A/extracellular signal-regulated kinase pathway. Mol Cell Biochem 2010; 338:115–122.

4 Merlotti D, Gennari L, Dotta F, Lauro D, Nuti R. Mechanisms of impaired bone strength in type 1 and 2 diabetes. Nutr Metab Cardiovasc Dis 2010; 20:683–690.

5 Moester MJ, Papapoulos SE, Löwik CW, van Bezooijen RL. Sclerostin: current knowledge and future perspectives. Calcif Tissue Int 2010; 87:99–107.

6 García-Martín A, Rozas-Moreno P, Reyes-García R, Morales-Santana S, García-Fontana B, García-Salcedo JA, Muíoz-Torres M. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab 2012; 97:234–241.

7 Link DC. Osteocytes link bone maintenance to blood homeostasis. Blood 2013; 121:867–868.

8 Dame C, Sutor AH. Primary and secondary thrombocytosis in childhood. Br J Haematol 2005; 129:165–177.

9 American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2005; 28(Suppl 1):S37–S42.

10 Berg KO, Maki BE, Williams JI, Holliday PJ, Wood-Dauphinee SL. Clinical and laboratory measures of postural balance in an elderly population. Arch Phys Med Rehabil 1992; 73:1073–1080.

11 Schoene D, Wu SM, Mikolaizak AS, Menant JC, Smith ST, Delbaere K, Lord SR. Discriminative ability and predictive validity of the timed up and go test in identifying older people who fall: systematic review and meta-analysis. J Am Geriatr Soc 2013; 61:202–208.

12 Spivak JL, Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis, and primary myelofibrosis: an alternative proposal. Blood 2008; 112:231–239.

13 Kanis JA. Assessment of fracture risk and its application to screening for postmenopausal osteoporosis: synopsis of a WHO report. WHO Study Group. Osteoporos Int 1994; 4:368–381.

14 García-Martín A, Reyes-García R, Rozas-Moreno P, Varsavsky M, Luque-Fernández I, Avilés-Pérez MD, *et al.* Variables which influence concentrations of sclerostin in patients with diabetes mellitus type 2 and its association with bone metabolism. Rev Osteoporos Metab Miner 2012; 4:109–116.

15 Chin C-W, Lin C-L, Gong ST. Increased bone mineral density with reduced body weight after discontinuation of pioglitazone in a lean type 2 diabetic male patient – a case report. Formos J Endocrin Metab 2012; 3:14–19.

16 Thrailkill KM, Lumpkin CK Jr, Bunn RC, Kemp SF, Fowlkes JL. Is insulin an anabolic agent in bone? Dissecting the diabetic bone for clues. Am J Physiol Endocrinol Metab 2005; 289:E735–E745.

17 Saller A, Maggi S, Romanato G, Tonin P, Crepaldi G. Diabetes and osteoporosis. Aging Clin Exp Res 2008; 20:280–289.

18 Tuominen JT, Impivaara O, Puuka P, Ronnemaa T. Bone mineral density in patients with type 1 and type 2 diabetes. Diabetes Care 1999; 22:1196–1200.

19 Maghbooli ZH, Hossein-nezhad A, Khoshniat M, Adibi H, Mohammadzadeh N, Larijani B. A study of bone mineral density in diabetes mellitus patients. Iran J Public Health 2007; 0:37–44.

20 Veldhuis-Vlug AG, Fliers E Bisschop PH. Bone as a regulator of glucose metabolism. Neth J Med 2013; 71:396–400.

21 Janghorbani M, Van Dam RM, Willett WC, Hu FB. Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture. Am J Epidemiol 2007; 166:495–505.

22 Schwartz AV, Vittinghoff E, Bauer DC, Hillier TA, Strotmeyer ES, Ensrud KE, *et al.* Association of BMD and FRAX score with risk of fracture in older adults with type 2 diabetes. JAMA 2011; 305:2184–2192.

23 Strotmeyer ES, Cauley JA, Schwartz AV, Nevitt MC, Resnick HE, Bauer DC, *et al.* Non traumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults: the health, aging, and body composition study. Arch Intern Med 2005; 165:1612–1617.

24 Schurman L, McCarthy AD, Sedlinsky C, Gangoiti MV, Arnol V, Bruzzone L, Cortizo AM. Metformin reverts deleterious effects of advanced glycation end-products (AGEs) on osteoblastic cells. Exp Clin Endocrinol Diabetes 2008; 116:333–340.

25 Kang L, Chen Q, Wang L, Gao L, Meng K, Chen J, *et al.* Decreased mobilization of endothelial progenitor cells contributes to impaired neovascularization in DM. Clin Exp Pharmacol Physiol 2009; 36:e47–e56.

26 Sorva A, Tilvis RS. Low serum ionized to total calcium ratio: association with geriatric DM and with other cardiovascular risk factors? Gerontology 1990; 36:212–216.

27 Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB. Vitamin D and calcium intake in relation to type 2 diabetes in women. Diabetes Care 2006; 29:650–656.

28 Abbas WA, Al-Zubaidi MA, Al-Khazraji SK. Estimation of serum calcium and parathyroid hormone (PTH) levels in diabetic patients in correlation with age and duration of disease. Iraq J Comm Med 2012; 2:161–164.

29 Paula FJ, Lanna CM, Shuhama T, Foss MC. Effect of metabolic control on parathyroid hormone secretion in diabetic patients. Braz J Med Biol Res 2001; 34:1139–1145.

30 Hofbauer LC, Brueck CC, Singh SK, Dobnig H. Osteoporosis in patients with diabetes mellitus. J Bone Miner Res 2007;22:1317–1328.

31 McNair P, Madsbad S, Christiansen C, Christensen MS, Transbøl I. Hyponatremia and hyperkalemia in relation to hyperglycemia in insulin-treated diabetic out-patients. Clin Chim Acta 1982; 120:243–250.

32 Starup-Linde J. Diabetes, biochemical markers of bone turnover, diabetes control, and bone. Front Endocrinol (Lausanne) 2013; 4: :21.

33 Parkinson IH, Fazzalari NL. Interrelationships between structural parameters of cancellous bone reveal accelerated structural change at low bone volume. J Bone Miner Res 2003; 18:2200–2205.

34 Leslie WD, Rubin MR, Schwartz AV, Kanis JA. Type 2 diabetes and bone. J Bone Miner Res 2012; 27:2231–2237.

35 Gennari L, Merlotti D, Valenti R, Ceccarelli E, Ruvio M, Pietrini MG, *et al.* Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. J Clin Endocrinol Metab 2012; 97:1737–1744.

36 Van Lierop AH, Hamdy NA, van der Meer RW, Jonker JT, Lamb HJ, Rijzewijk LJ, *et al.* Distinct effects of pioglitazone and metformin on circulating sclerostin and biochemical markers of bone turnover in men with type 2 diabetes mellitus. Eur J Endocrinol 2012; 166:711–716.

37 Polyzos SA, Anastasilakis AD, Bratengeier C, Woloszczuk W, Papatheodorou A, Terpos E. Serum sclerostin levels positively correlate with lumbar spinal bone mineral density in postmenopausal women – the six-month effect of risedronate and teriparatide. Osteoporos Int 2012; 23:1171–1176.

38 Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, *et al.* Bone dysplasia sclerosteosis results from loss of the *SOST* gene product, a novel cystine knot-containing protein. Am J Hum Genet 2001; 68:577–589.

39 Balemans W, Patel N, Ebeling M, Van Hul E, Wuyts W, Lacza C, *et al.* Identification of a 52 kb deletion downstream of the *SOST* gene in patients with van Buchem disease. J Med Genet 2002; 39:91–97.

40 Ma L, Oei L, Jiang L, Estrada K, Chen H, Wang Z, *et al.* Association between bone mineral density and type 2 diabetes mellitus: a meta-analysis of observational studies. Eur J Epidemiol 2012; 27:319–332.

41 Mödder UI, Hoey KA, Amin S, McCready LK, Achenbach SJ, Riggs BL, *et al.* Relation of age, gender, and bone mass to circulating sclerostin levels in women and men. J Bone Miner Res 2011; 26: 373–379.

42 Vestergaard P. Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes – a meta-analysis. Osteoporos Int 2007; 18:427–444.

43 Strotmeyer ES, Cauley JA, Schwartz AV, Nevitt MC, Resnick HE, Zmuda JM, *et al.* Health ABC Study. Diabetes is associated independently of body composition with BMD and bone volume in older white and black men and women: the Health, Aging, and Body Composition Study. J Bone Miner Res 2004; 19:1084–1091.

44 Andress DL, Hercz G, Kopp JB, Endres DB, Norris KC, Coburn JW, *et al.* Bone histomorphometry of renal osteodystrophy in DM patients. J Bone Miner Res 1987; 2:525–531.

45 Fulzele K, Krause DS, Panaroni C, Saini V, Barry KJ, Liu X, *et al.* Myelopoiesis is regulated by osteocytes through Gs alpha-dependent signaling. Blood 2013; 121:930–939.

46 Vani S, Mohen V. Essential primary thrombocytosis in a diabetic patient. Asian J Diabetol 2005; 7:42–45.