Variables which influence concentrations of sclerostin in patients with diabetes mellitus type 2 and its association with bone metabolism

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Summary

Background and objectives: Diabetes mellitus type 2 (DM2) is associated with an increased risk of fractures whose underlying mechanisms are complex. The objective of this study was to analyse the variables which influence blood concentrations of sclerostin and the relationship with bone metabolism in a group of DM2 patients.

Patients and methods: A transversal study of 76 patients with DM2. Clinical data, basic biochemical parameters, calcitropic hormones, markers for bone remodelling, vertebral X-rays and bone mineral density (BMD) were gathered. Blood concentrations of sclerostin were determined using ELISA (Biomedica, Austria).

Results: The males had higher concentrations than the females (63.15±27.03 vs 43.14±17.08 pmol/L, p<0.001). We found positive relationships between sclerostin and age in males with DM2 (r=0.338, p=0.031) and between sclerostin and creatinine in the whole sample (adjusted for age: r=0.362, p<0.001). Also, it had a negative relationship with bone alkaline phosphatase (BAP) (r=-0.259, p=0.029), carboxy-terminal telopeptide of type 1 collagen (CTX) (r=-0.356, p=0.002) and tartrate-resistant acid phosphatase 5β (TRAP5β) (r=-0.289, p=0.013). BMD in the lumbar spine, femoral neck and total hip were positively associated with sclerostin (r=0.373, r=0.492, r=0.524, p<0.001) adjusted for age. Blood levels of sclerostin were lower in patients with DM2 and osteoporosis than those who were non-osteoporotic (42.96±19.16 vs 56.95±25.98 pmol/L, p=0.041).

Conclusions: Sex, age and renal function are determining factors of levels of sclerostin in the circulation of patients with DM2. There is a negative relationship with remodelling markers and a positive one with BMD. Blood levels of sclerostin are lower in patients with DM2 and osteoporosis.

Key words: sclerostin, diabetes mellitus type 2, bone metabolism.

Abbreviations: FN: femoral neck; LS: lumbar spine; TH: total hip; CTX: carboxy-terminal telopeptide of type 1 collagen; DXA: dual X-ray absorptiometry; BAP: bone alkaline phosphatase; GF: glomerular filtration; BBG: basal blood glucose; HbA1c: glycedated haemoglobin; BMI: body mass index; PTH: parathormone; OC: osteocalcin; TRAP5β: tartrate-resistant acid phosphatase 5β; 25(OH)D: 25-hydroxyvitamin D.
Introduction
Osteoporosis and diabetes mellitus are two highly prevalent diseases which are associated with an increased risk of fragility fractures, and a substantial impact on the morbidity and mortality of the general population. While various observational studies have investigated the association between the two, the mechanism by which diabetes favours the appearance of fractures does not appear to have been established adequately. The discovery of the Wnt pathway, which stimulates the differentiation of osteoblast precursors, has meant a recent advance in the understanding of bone homeostasis. Thus, the role of this signalling pathway and its antagonists may be crucial in the pathogenesis of the alterations in bone quality seen in diabetes mellitus.

The data published from animal experiments centre on an analysis of gene expression and the concentration in the bone microenvironment of some the proteins involved. In fact, a study in mice with diabetes mellitus type 1 (DM1) induced by streptozotocin showed suppression of the gene expression for sclerostin, an increase in osteocyte apoptosis and low concentrations of total and nuclear β-catenin. On the other hand, Nuche-Berenguer et al. showed that the gene expression for Dkk1 and SOST in models of mice with DM2 were found to be suppressed, while in models of insulin-resistant mice there was evidence of gene overexpression of SOST associated with an increase in levels of mRNA for LRP5.

Previously, we have reported that levels of sclerostin were found to be raised in patients with diabetes mellitus type 2 (DM2), coinciding with the findings of Nuti et al. The aim of our study was to analyse the variables which influence blood concentrations of sclerostin and the relationship with bone metabolism in a group of patients with DM2.

Patients and methods
Study population
Our study, of a transversal nature, included a group of patients with DM2 diagnosed according to the criteria of the American Diabetes Association. They were recruited consecutively from January 2006 to December 2007 in the endocrinology and nutrition clinic of the University Hospital San Cecilio of Granada.

All the patients met the following inclusion criteria: Caucasian, mobile, aged between 35 and 65 years and with normal haemogram, creatinine, liver function, calcium and phosphorus values. The exclusion criteria were: chronic disease except DM2, conditions which affect bone metabolism (Paget’s disease, rheumatoid arthritis, hyperparathyroidism, hypercortisolism, malignant tumours, transplant) and treatment with drugs which interfere in bone metabolism (causal supplements, vitamin D preparations, selective estrogen receptor modulators, calcitomin, estrogen therapy, antiresorptives, thiazides, glucocorticoids or anticonvulsants).

The study was carried out with the approval of the ethics committee of the hospital and adjusted according to the relevant directives for research in humans. All the patients signed their informed consent for their inclusion.

Analytical determinations
Basal blood glucose (BBG), glycated haemoglobin (HbA1c), calcium, phosphorus and creatinine were measured using automated laboratory techniques. The glomerular filtrate rate (GF) was estimated using the Cockcroft-Gault equation. The blood levels of parathormone (PTH immunoassay, Roche Diagnostics SL, Bracelona, Spain) and 25-hydroxyvitamin D (25-OH-D, radioimmunoassay, Diasorin, Stillwater, Minnesota, US).

The remodelled bone markers for formation collected were: osteocalcin (OC, radioimmunoassay, Diasorin, Minnesota, US) and bone alkaline phosphatase (BAP) (BAP, ELISA, Tandem-R Ostase TM, Hybritech Europe, Liege, Belgium). The markers for resorption were: tartrate-resistant acid phosphatase 5β (TRAPβ, colourimetry, Hitachi 704 Boehringer Mannheim GmbH) and carboxy-terminal telopeptide of type I collagen (CTX, enzymatic immunoassay, analyser Elecsys Crosslaps, Roche Diagnostics SL, Barcelona, Spain).

Blood levels of sclerostin were measured using ELISA (Biomedica, Austria). In our laboratory, two samples with a known concentration were tested 6 times to calculate the intratrial variability which was 4% and two samples of known concentration were tested to calculate the intertrial variability which was 3%. The measurement of sclerostin was expressed in picomoles per litre (pmol/l) and the minimum detection level was <10 pmol/l.

Bone mineral density and vertebral X-ray study
The bone mineral density (BMD) of the lumbar spine (LS) L2-L4, femoral neck (FN) and total hip (TH) were determined in all patients using dual X-ray absorptiometry (DXA) using the Hologic® QDR-4500 densitometer (Whatman, MA; coefficient of variation <1%). All the measurement were made by the same operator. We used the criteria of the World Health Organisation (WHO) for the diagnosis of osteoporosis. Simple X-rays (XR) were also carried out of the dorsal and lumbar spine for the analysis of morphometric vertebral fractures and were interpreted in accordance with the algorithm developed by Genant et al.

Statistical analysis
The statistical analysis of the data was carried out using the SPSS programme (version 15.0, Chicago, US). For continuous variables, the Kolmogorov-Smirnoff test was used to evaluate if they would follow a normal distribution. Measures of central tendency (mean) and dispersion (standard deviation, range) for continuous variables, and distribution of absolute and relative frequencies for categorical variables, were used. The differences for the variables of interest between com-
Table 1. Characteristics of the study sample

<table>
<thead>
<tr>
<th></th>
<th>n=76</th>
<th>Men n=41</th>
<th>Women n=35</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>57.9±6.5</td>
<td>57.4±6.8</td>
<td>58.6±6.1</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>31.3±5.7</td>
<td>29.8±4.4</td>
<td>33±6.6</td>
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<tr>
<td>Duration diabetes (years)</td>
<td>13.4±7.5</td>
<td>13.2±6.7</td>
<td>13.6±8.5</td>
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<td>Serum parameters:</td>
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<td></td>
<td></td>
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<tr>
<td>- BBG (mg/dL)</td>
<td>174.8±62.9</td>
<td>177±65.3</td>
<td>172.3±60.9</td>
</tr>
<tr>
<td>- HbA1c (%)</td>
<td>8±1.9</td>
<td>8.1±2</td>
<td>7.9±1.8</td>
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<td>- Creatinine (mg/dL)</td>
<td>0.9±0.2</td>
<td>0.8±0.3</td>
<td>1±0.1</td>
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<td>- GFO (ml/min/1.73 m²)</td>
<td>93.4±26.9</td>
<td>95.8±29.5</td>
<td>92.3±24.3</td>
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<tr>
<td>- Calcium (mg/dL)</td>
<td>9.6±0.5</td>
<td>9.6±0.5</td>
<td>9.4±0.5</td>
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<tr>
<td>- Phosphorus (mg/dL)</td>
<td>3.7±0.6</td>
<td>3.6±0.6</td>
<td>3.8±0.4</td>
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<td>- PTH (pg/mL)</td>
<td>38.7±18.4</td>
<td>33.5±15.2</td>
<td>43.8±20</td>
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<td>- 25(OH)D (ng/mL)</td>
<td>17.6±11.2</td>
<td>17.6±10.1</td>
<td>18.1±12.5</td>
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<td>- OC (ng/mL)</td>
<td>1.45±1.27</td>
<td>1.35±1.19</td>
<td>1.62±1.33</td>
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<td>- BAP (µg/L)</td>
<td>14.8±6.5</td>
<td>13.4±4.2</td>
<td>16.6±8.3</td>
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<td>- CTX (ng/mL)</td>
<td>0.212±0.13</td>
<td>0.163±0.082</td>
<td>0.26±0.14</td>
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<td>- TRAP5β (UI/L)</td>
<td>1.38±1</td>
<td>1.26±0.96</td>
<td>1.56±1.02</td>
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<td>- Sclerostin (pmol/L)</td>
<td>53.9±24.95</td>
<td>63.15±27.03</td>
<td>43.14±17.08</td>
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<td>Parameters DXA:</td>
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<td>- BMD CL (g/cm²)</td>
<td>0.949±0.142</td>
<td>0.963±0.131</td>
<td>0.952±0.153</td>
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<td>- BMD CF (g/cm²)</td>
<td>0.818±0.13</td>
<td>0.861±0.131</td>
<td>0.765±0.109</td>
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<td>- BMD CT (g/cm²)</td>
<td>0.905±0.142</td>
<td>0.942±0.145</td>
<td>0.859±0.127</td>
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<td>- T-score CL</td>
<td>-1.3±1.3</td>
<td>-1.375±1.218</td>
<td>-1.317±1.452</td>
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<td>- T-score CF</td>
<td>-0.59±1</td>
<td>-0.461±1.052</td>
<td>-0.758±0.981</td>
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<tr>
<td>- T-score CT</td>
<td>-0.61±1</td>
<td>-0.508±1</td>
<td>-0.661±1.03</td>
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<tr>
<td>Osteoporosis (%)</td>
<td>19.7</td>
<td>9.2</td>
<td>10.5</td>
</tr>
<tr>
<td>Vertebral fractures (%)</td>
<td>26.3</td>
<td>18.4</td>
<td>7.9</td>
</tr>
</tbody>
</table>

BMI: body mass index; BBG: baseline blood glycemia; HbA1c: glycated haemoglobin; GFO: glomerular filtration index; PTH: parathormone; 25(OH)D: 25-hydroxyvitamin D; OC: osteocalcin; BAP: bone alkaline phosphatase; CTX: carboxy-terminal telopeptide of type 1 collagen; TRAP5β: tartrate-resistant acid phosphatase 5β; BMD: bone mineral density; LS: lumbar spine; FN: femoral neck; TH: total hip.

Comparison groups were carried out using Student's t-test for two independent samples and the Mann-Whitney U-test in the case of continuous variables. For categorical variables Pearson's chi squared test and Fisher's exact test were used. The relationship between the quantitative variables was analysed using Pearson's or Spearman's bivariate correlation test. To control the effect of one or more variables on the Pearson correlation coefficient the partial correlation test was used. All the statistical tests were carried out as two-tailed. A value of p<0.05 was considered to be statistically significant.

Results
Table 1 shows the clinical, biochemical and densitometric characteristics of the whole sample, and according to sex. The diabetic women had a body mass index (p=0.016), blood levels of PTH (p=0.01) and CTX (p<0.001) greater than the men,
while the men had a higher BMD in the femoral neck (p=0.002) and in the total hip (p=0.015) compared with the women. There were no differences in the rest of the variables.

The men had higher concentrations of sclerostin than the women (63.15±27.03 as opposed to 43.14±17.08 pmol/L, p<0.001) (Table 1 and Figure 1). In the males, the levels of sclerostin were positively correlated with age (r=0.333, p=0.031), but this relationship did not hold for women (r=0.223, p=0.213) (Figure 2).

In the total sample blood levels of sclerostin showed a positive correlation with values of creatinine in the blood (r=0.37, p<0.001) and negative, although not statistically significantly, with glomerular filtrate (r=−0.184, p>0.05). After adjusting for age this relationship remained significant for blood levels of creatinine (r=0.361, p=0.001). The levels of sclerostin were negatively correlated with the marker for bone formation BAP (r=−0.277, p=0.021) and with the markers for bone resorption CTX (r=−0.363, p=0.002) and TRAPβ (r=−0.276, p=0.02). There was no relationship with the marker for formation OC (Figure 3).

BMD and T-score in the lumbar spine, femoral neck and total hip were positively related with levels of sclerostin after adjusting for age (Table 2).

In patients with osteoporosis the levels of sclerostin were significantly lower than the non-osteoporotic patients (44.03±19.41 pmol/L as opposed to 56.95±25.98 pmol/L, p=0.048) (Figure 4). However there was no relationship with morphometric fractures (54.03±26.55 as opposed to 53.72±23.27 pmol/L, p>0.05).

**Discussion**

The levels of sclerostin were found to be increased in males. These results coincide with those described in a broad populational cohort study of 362 women and 318 men in which the women, whether they were pre- or postmenopausal, had lower levels of sclerostin than the men. The authors postulate that the larger size of the skeleton, around 21% greater in males, may explain the gender differences in blood concentrations of sclerostin. On the other hand, Mödder et al. maintain that the estrogens influence and regulate the synthesis of sclerostin, basing the differences observed on the levels of sclerostin among pre- and postmenopausal women, these being lower in the former, and on an earlier study in which treatment with estrogens in postmenopausal women reduced levels of sclerostin by 27%.

Molecular studies support the estrogenic role in the regulation of bone mass through the Wnt pathway by means of the α-estrogen receptor which is involved in the transport to the nucleus of β-catenin in response to the mechanical tension of the osteocyte.

We observed an increase in blood levels of sclerostin with age in males. The influence of age on levels of sclerostin is being studied in depth. It is known that the expression of the Wnt pathway proteins by the osteoblast are regulated individually by age, and a number of clinical studies have confirmed this relationship in both men and women. Hence, a population study carried out in 1,235 premenopausal, and 568 postmenopausal women in an age range from 20 to 79 years, analysed the changes in blood concentrations of sclerostin with age. One of the conclusions was that between 35 and 45 years of age the levels of sclerostin remained stable, and from 45 years of age they increased progressively. Some authors postulate that the production of sclerostin in each osteocyte increases with age, while not excluding the possibility that their clearance is reduced.

Although the way sclerostin is eliminated is not known, the most probable option, given the size and weight of this protein, is that it is eliminated in the kidney. In our study the levels of sclerostin
were positively related with blood concentrations of creatinine and negatively with glomerular filtrate. These results coincide with earlier works which show that levels of sclerostin increase with a deterioration of renal function, above all in chronic renal insufficiency grade 3 or higher, and with no relationship to hepatic function\(^1\). Similarly, in patients with chronic renal disease in haemodialysis levels of sclerostin are higher than those of controls\(^1\).

In theory, raised concentrations of sclerostin ought to be associated with a decrease in markers for bone formation. However, we found that levels of sclerostin in patients with diabetes were negatively related with both markers for formation (BAP\(^9\)) and resorption (CTX and TRAP). Similarly, in women over the age of 60 years levels of sclerostin were negatively associated with blood levels of BAP and amino-terminal propeptide of type 1 collagen (P1NP), as well as CTX. In patients immobilised after a stroke blood sclerostin was negatively correlated with BAP and positively with CTX\(^1\). On the other hand, other studies found no relationship between sclerostin and markers for bone remodelling\(^1\). Therefore, we consider that the data regarding sclerostin and markers for bone remodelling are contradictory and do not allow definitive conclusions to be drawn.

Bone mass, expressed as BMD, T-score and Z-score, are positively related with levels of sclerostin, both in the group with DM2 as well as in the control group in our study. Similarly, BMD was the main predictive variable for blood concentration of sclerostin. These findings differ from those observed in patients with sclerosteosis or Van Buchem’s disease\(^2\) and in Knockout mice models for sclerostin or with overexpression of sclerostin\(^2\). Given that the physiological role of sclerostin is the inhibition of osteoblast proliferation and activity, what would be expected would be a negative relationship with bone mass. However, our results coincide with some other works in which this aspect is examined. Thus, in patients with renal insufficiency in haemodialysis levels of sclerostin were positively correlated both with BMD in the femoral neck, lumbar spine and radius, as well as with trabecular density and the number of trabeculae in the radius and tibia measured using high resolution peripheral computerised tomography (CT)\(^9\). In addition, the BMD and BMC (bone mineral content) in the lumbar spine and hip were positively related with the concentration of sclerostin in healthy subjects after adjusting for age, sex and renal function\(^9\). In the cohort of Mödder et al. a positive association was also found between total BMC and levels of sclerostin, but only significant from 40 years of age, and greater from 60 years of age\(^9\). Also, the levels of sclerostin were related positively with BMD in the distal femur and proximal tibia in patients with chronic medullar lesion\(^9\).

In agreement with earlier findings, levels of sclerostin were lower in patients with diabetes and
densitometric osteoporosis compared with those patients with diabetes but without densitometric osteoporosis. A similar relationship has been described between osteoporosis and sclerostin in women with postmenopausal osteoporosis to that which we found in patients with DM219.

Various hypotheses have been suggested to explain the positive relationship between blood sclerostin and bone mass. The main one is that there are changes in the production of sclerostin by the osteocytes in relation to aging, with a higher production by each individual osteocyte9. On the other hand, the increase in the levels of sclerostin means a decrease in bone formation on the basis of its physiological functions, and therefore, allows there to be a drop in bone turnover. A lower bone turnover would mean slowed bone loss and higher bone mass16.

In summary, sex, age and renal function are determining factors in blood levels of sclerostin in patients with DM2. Similarly, we found a negative relationship with markers for bone remodelling and a positive relationship with BMD. Finally, blood levels of sclerostin are lower in patients with DM2 and osteoporosis, with no relationship to the presence of fractures.

Conflict of interest: There are no conflicts of interest on the part of the authors.

Bibliography

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