CLINICAL STUDY

Distinct effects of pioglitazone and metformin on circulating sclerostin and biochemical markers of bone turnover in men with type 2 diabetes mellitus

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Abstract

Objective: Patients with type 2 diabetes mellitus (T2DM) have an increased risk of fractures and thiazolidinediones (TZDs) increase this risk. TZDs stimulate the expression of sclerostin, a negative regulator of bone formation, *in vitro*. Abnormal sclerostin production may, therefore, be involved in the pathogenesis of increased bone fragility in patients with T2DM treated with TZDs.

Methods: We measured serum sclerostin, procollagen type 1 amino-terminal propeptide (P1NP), and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) in 71 men with T2DM treated with either pioglitazone (PIO) (30 mg once daily) or metformin (MET) (1000 mg twice daily). Baseline values of sclerostin and P1NP were compared with those of 30 healthy male controls.

Results: Compared with healthy controls, patients with T2DM had significantly higher serum sclerostin levels (59.9 vs 45.2 pg/ml, $P < 0.001$) but similar serum P1NP levels (33.6 vs 36.0 ng/ml, $P = 0.39$). After 24 weeks of treatment, serum sclerostin levels increased by 11% in PIO-treated patients and decreased by 1.8% in MET-treated patients ($P = 0.018$). Changes in serum sclerostin were significantly correlated with changes in serum CTX in all patients ($r = 0.36$, $P = 0.002$) and in PIO-treated patients ($r = 0.39$, $P = 0.020$), but not in MET-treated patients ($r = 0.17$, $P = 0.31$).

Conclusions: Men with T2DM have higher serum sclerostin levels than healthy controls, and these levels further increase after treatment with PIO, which is also associated with increased serum CTX. 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 to the detrimental effect of TZDs on bone.

European Journal of Endocrinology 166 711–716

Introduction

Patients with type 2 diabetes mellitus (T2DM) have an increased risk of fractures (1), but the mechanism(s) responsible for the increased bone fragility remain unclear. Moreover, blood glucose-lowering therapy using thiazolidinediones (TZDs) has also been reported to cause bone loss (2) and to further increase the risk of fractures (3, 4). Activation of PPAR γ and preferential stimulation of the differentiation of bone marrow mesenchymal stem cells into adipocytes at the expense of osteoblasts have been proposed as potential mechanisms for these effects of TZDs on bone (5). TZDs have also been recently reported to stimulate sclerostin synthesis *in vitro*, suggesting an additional mechanism for the detrimental effects of TZDs on bone (6). Sclerostin is a glycoprotein synthesized in bone by osteocytes, which reduces bone formation (7, 8), and serum sclerostin levels have been found to be

significantly correlated with fracture risk in postmenopausal women (9). Abnormal sclerostin production may contribute to the pathogenesis of bone fragility of patients with T2DM as well as in the actions of TZDs on bone. To test this hypothesis, we measured serum sclerostin and biochemical markers of bone turnover in men with T2DM before and after treatment with the TZD pioglitazone (PIO).

Materials and methods

We studied 71 men with T2DM who participated in the PIRAMID study (PIO Influence on Triglyceride Accumulation in the Myocardium in Diabetes). The design and results of this study (10) as well as other metabolic parameters of the patients including whole body insulin sensitivity (11) have been reported previously. In brief, this was a 24-week prospective,

randomized, double-blind, double-dummy with active comparator, two-center parallel group intervention study. Men with uncomplicated T2DM, aged 45–65 years, were included in the study. After a 10-week washout period of previous medications, patients were treated with glimepiride for 8 weeks and were subsequently randomized to PIO (15 mg once daily, titrated to 30 mg once daily after 2 weeks) or metformin (MET) (500 mg twice daily, titrated to 1000 mg twice daily) and matching placebo to be taken in addition to glimepiride throughout the study. Blood samples were collected after an overnight fast at baseline and after 24 weeks of treatment and were stored at -80°C until assayed.

Baseline data including serum sclerostin and P1NP values were compared with those of 30 previously described healthy male volunteers (12). All these subjects had normal serum calcium concentrations, renal function, and bone turnover markers and were not using medications that could affect calcium or bone metabolism.

The study was approved by the medical ethics committees of the two participating centers, and informed consent was obtained from all the participants in the study.

Serum biochemistry

Sclerostin was measured by a highly sensitive electrochemiluminescence assay (Mesoscale Discoveries, Gaithersburg, MD, USA) as described previously (13). The intraprecision of the assay was 6% and the interprecision 10%. All samples from individual patients were measured in the same assay. Serum calcium adjusted for albumin binding, phosphate, and creatinine were measured by semiautomated techniques. Procollagen type 1 amino-terminal propeptide (P1NP) and carboxy-terminal cross-linking telopeptide of type I collagen (CTX) were measured by the E-170 system (Roche BV).

Statistical analysis

Data are expressed as mean $\pm$ s.d. (unless otherwise stated). Absolute changes and percentage changes in biochemical parameters between baseline and end-of-study values were calculated for each subject. Differences between groups were assessed by Student's *t*-tests. Differences in sclerostin levels between patients and healthy controls were corrected for BMI and weight using a linear mixed model. Within-treatment group differences in percentage changes in measured parameters were tested by one-sample *t*-test. Correlations were assessed by Pearson's correlation tests. Statistical analysis was performed using the SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). A *P* value of <0.05 was considered to be statistically significant.

Results

Baseline

The mean age of patients with T2DM (56.5 ± 5.6 years) was comparable with that of controls (55.0 ± 16.4 years, $P=0.50$), but patients were overweight (patients: 92.2 ± 13.6 kg, controls: 80.7 ± 12.1 kg, $P<0.001$) and had a higher BMI (patients: 28.7 ± 3.4 kg/m², controls: 25.4 ± 4.0 kg/m², $P<0.001$) (Table 1).

Patients with T2DM had significantly higher serum sclerostin levels compared with healthy controls (59.9 pg/ml; 95% CI: 55.2 to 64.8 and 45.2 pg/ml; 95% CI: 40.6 to 49.8 pg/ml, $P<0.001$) (Fig. 1A). This difference in sclerostin levels remained significant after adjusting for BMI or weight ($P=0.008$). In patients with T2DM, serum sclerostin levels were positively correlated with serum fasting insulin levels ($r=0.41$, $P<0.001$), but not with HbA1c ($r=0.07$, $P=0.57$), weight ($r=0.07$, $P=0.58$), or BMI ($r=0.18$, $P=0.12$). There was no difference in mean serum P1NP levels between patients and controls (33.6 ng/ml; 95% CI: 31.0 to 36.6 ng/ml and 36.0 ng/ml; 95% CI: 30.7 to 40.8 ng/ml respectively, $P=0.39$) (Fig. 1B).

Response to treatment

There was no significant difference in age, weight, and time since diagnosis of T2DM between the two treatment groups (Table 2). Baseline values and changes in plasma glucose, insulin, and HbA1c after 24 weeks of treatment have been previously reported (10). Baseline values were similar in the two treatment groups and improved significantly, resulting in a similar degree of glycemic control during the 24 weeks of treatment with either MET or PIO.

Baseline values of serum sclerostin and of biochemical markers of bone turnover and calcium metabolism were similar between the two treatment groups (Table 2). The two treatment regimens had, however, different effects on serum sclerostin and bone marker levels (Fig. 2). Serum sclerostin levels increased by 11% (95% CI: 2.26 to 19.8, $P=0.019$) after 24 weeks of treatment with PIO, but there was no

Table 1 Baseline anthropometric and biochemical data of male patients with T2DM and healthy controls. Values are given as mean $\pm$ s.d.

	Controls (<i>n</i> =30)	Patients (<i>n</i> =71)	<i>P</i> value
Age (years)	55.0 ± 16.4	56.5 ± 5.6	0.59
Weight (kg)	80.7 ± 12.1	92.2 ± 13.6	<0.001
BMI (kg/m ²)	25.4 ± 4.0	28.6 ± 3.3	<0.001
Sclerostin (pg/ml)	45.2 ± 12.8	59.2 ± 19.4	<0.001
P1NP (ng/ml)	36.0 ± 13.8	33.8 ± 12.2	0.43
CTX (pg/ml)	–	311 ± 14	
Calcium (mmol/l)	2.27 ± 0.10	2.29 ± 0.10	0.36
Phosphate (mmol/l)	1.05 ± 0.12	0.99 ± 0.17	0.41

Figure 1 Serum sclerostin (A) and P1NP (B) levels in male patients with T2DM and healthy male controls. Bars represent s.e.m. *P* values: significance of differences between groups (Student's *t*-tests). NS, nonsignificant.

significant change in these levels in patients treated with MET (-1.8% ; 95% CI: -8.8 to 5.0 , $P=0.42$). The difference in sclerostin response between the two treatment groups was significant ($P=0.018$).

Serum P1NP levels increased by 3.7% (95% CI: -3.9 to 11.3 , $P=0.35$) in the PIO group but decreased significantly by 19.1% (95% CI: -25.5 to -12.8 , $P<0.001$) in the MET group (between-group difference $P<0.001$). Serum CTX levels increased by 16.8% (95% CI: 4.4 to 29.1 , $P=0.012$) after treatment with PIO but decreased by 19.0% (95% CI: -27.1 to -10.9 , $P<0.001$) in the MET-treated group (between-group difference $P<0.001$). There was no significant change in serum calcium, phosphate, or creatinine concentrations after 24 weeks of treatment with either agent.

Relationship between serum sclerostin and markers of bone turnover

There was no significant relationship between serum sclerostin levels and either serum P1NP ($r=0.15$, $P=0.22$) or serum CTX ($r=0.17$, $P=0.17$) at baseline.

There was also no significant correlation between changes in serum sclerostin values and serum P1NP levels in all patients pooled together ($r=0.12$, $P=0.31$), or in individual treatment groups (MET: $r=0.02$, $P=0.90$; PIO: $r=0.05$, $P=0.77$) (Fig. 3A). In contrast, changes in serum sclerostin levels were significantly correlated with changes in serum CTX levels in all patients pooled together ($r=0.36$, $P=0.002$) and in PIO-treated patients ($r=0.39$, $P=0.020$), but not in the MET-treated patients ($r=0.17$, $P=0.31$) (Fig. 3B).

Discussion

We here show that circulating sclerostin levels are increased in patients with uncomplicated T2DM and respond differently to different blood glucose-lowering medications. In the presence of similar control of glycemia, MET treatment given for 24 weeks had no effect on sclerostin levels and significantly decreased levels of biochemical markers of bone turnover, whereas PIO treatment increased the serum levels of sclerostin and the bone resorption marker CTX.

The exact pathogenesis of increased bone fragility observed in patients with T2DM remains unclear. In these patients, it is well established that fractures occur at higher bone mineral density (BMD) values than in patients with osteoporosis. The increased fracture rate has also been found to be independent of the increased frequency of falls, also documented in patients with T2DM (1, 14, 15). Changes in calcium homeostasis, increased secretion of inflammatory cytokines, and the accumulation of advanced glycation end-products have been proposed as contributory factors to the pathogenesis of increased bone fragility in T2DM (16, 17). The identification of osteocyte-produced sclerostin as a key regulator of bone formation by osteoblasts has initiated a number of studies on the effect of this protein on bone strength. Lack of sclerostin leads to profound increases in bone mass in humans (13, 18) and in animal models (19), whereas overexpression of

Table 2 Baseline anthropometric and biochemical data of male patients with T2DM treated with either metformin or pioglitazone. Values are given as mean $\pm$ s.d.

	Metformin (<i>n</i> =37)	Pioglitazone (<i>n</i> =34)	<i>P</i> value
Age (years)	56.5 $\pm$ 5.4	56.4 $\pm$ 5.9	0.99
Weight (kg)	93.2 $\pm$ 14.6	91.1 $\pm$ 12.6	0.50
BMI (kg/m ²)	29.3 $\pm$ 3.8	28.2 $\pm$ 3.0	0.17
Time since diagnosis (years)	3.6 $\pm$ 2.5	4.6 $\pm$ 2.8	0.11
Sclerostin (pg/ml)	62.4 $\pm$ 18.9	58.5 $\pm$ 22.0	0.42
P1NP (ng/ml)	35.1 $\pm$ 11.7	32.0 $\pm$ 12.7	0.28
CTX (pg/ml)	381 $\pm$ 380	294 $\pm$ 140	0.40
Calcium (mmol/l)	2.29 $\pm$ 0.10	2.28 $\pm$ 0.11	0.92
Phosphate (mmol/l)	1.00 $\pm$ 0.16	1.00 $\pm$ 0.18	0.60
Creatinine (μ mol/l)	76.6 $\pm$ 17.3	77.3 $\pm$ 12.7	0.49

Figure 2 Mean percentage changes in serum levels of sclerostin, P1NP, and CTX in male patients with T2DM treated with metformin (open circles) or pioglitazone (closed squares). Bars represent the 95% confidence interval. *Significance of change from baseline within groups (one-sample *t*-test), $P < 0.05$. *P* values: significance of differences between groups (Student's *t*-tests).

sclerostin is associated with decreased mechanical strength in animal models (8). In a recent study, circulating sclerostin levels were positively associated with fracture risk in a large cohort of postmenopausal women (9). Moreover, established determinants of bone strength such as mechanical loading, parathyroid hormone, and estrogen have been shown to modulate the production and/or the secretion of sclerostin (12, 20, 21). Our findings of increased serum sclerostin levels in patients with T2DM suggest a potential role for this protein in bone metabolism in T2DM by an as-yet unidentified mechanism. The positive correlation between serum sclerostin and insulin levels suggests a possible contribution of insulin in the production and/or secretion of sclerostin. However, in contrast to the case of osteoblasts, few or no insulin receptors could be detected in osteocytes by immunohistochemical staining (22). In our patients with T2DM, the documented increase in serum sclerostin levels was not associated with a decrease in serum levels of the bone formation marker P1NP, in agreement with a previous study (23). Another marker of bone formation, osteocalcin, particularly the undercarboxylated form, has been implicated in the regulation of insulin secretion (24). However, measurements of serum osteocalcin in patients with T2DM have provided conflicting results showing either no change or decrease (23, 25, 26, 27, 28, 29). We did not measure serum osteocalcin, but a significant positive relationship with serum P1NP has been previously reported in patients with T2DM (23, 28). Notwithstanding, our findings are in keeping with a recent study that reported higher serum sclerostin levels in patients with T2DM compared with controls but no difference in the bone formation markers serum osteocalcin and bone-specific alkaline phosphatase between T2DM patients and healthy controls (26).

In our cohort of T2DM patients, treatment with MET or PIO in combination with glimepiride improved glucose regulation to a similar degree but had different effects on serum sclerostin levels and biochemical markers of bone turnover. Treatment with MET for 24 weeks had no apparent effect on serum sclerostin levels, but significantly decreased bone turnover, as assessed by serum markers of bone formation (P1NP) and bone resorption (CTX). A decrease in bone turnover has been previously reported after MET treatment (30, 31), but a similar decrease in bone turnover has also been observed after improvement of glycemic control using other therapeutic regimens, such as diet or insulin administration (32). However, the different effects on bone metabolism observed in our study in the PIO-treated patients despite a similar control of glucose metabolism demonstrate that the two agents have different effects on bone metabolism that are independent of their glucose-lowering action.

Figure 3 Relationship between changes in serum levels of sclerostin and P1NP (A) and CTX (B). Open circles represent metformin-treated patients and closed squares represent pioglitazone-treated patients.

Our data indeed show that in contrast to MET, serum sclerostin levels increased in patients treated with PIO and that this was associated with a significant increase in serum CTX levels, despite adequate glycemic control. Over the past decade, evidence has been accumulating on the detrimental effect of TZDs on the skeleton, decreasing bone mass and increasing fracture risk in both men (33, 34, 35) and women with diabetes mellitus (4, 33, 35, 36, 37, 38, 39). This deleterious effect of TZDs on the skeleton is generally attributed to the activation of PPAR γ in the bone marrow by these agents, leading to preferential stimulation of adipogenesis at the cost of osteoblastogenesis (5). Our results suggest another potential pathogenetic mechanism, namely stimulation of sclerostin production by TZDs. This finding conforms to recent *in vitro* data by Mabileau *et al.* (6) who showed that TZDs stimulate the expression of sclerostin by osteocytes in the absence of 17 β -estradiol. Although sclerostin is a well-established inhibitor of bone formation, recent evidence indicates that it can also promote osteoclastogenesis by stimulating RANKL produced by osteocytes (40). Moreover, inhibition of sclerostin in animals and humans by a specific antibody does not only lead to increased bone formation but also to decreased bone resorption (41, 42). Taken together, these data suggest that PIO stimulates the production of sclerostin, which would in turn increase RANKL production by osteocytes, thus resulting in a dual effect on bone metabolism, reducing bone formation and increasing bone resorption, which could explain the adverse effect of PIO on bone quality.

A limitation of our study is that it was conducted only in men with uncomplicated T2DM and our results cannot be readily extrapolated to all patients with T2DM. The study was of short duration precluding the assessment of the effect of TZDs on BMD or fracture risk.

Notwithstanding, our study shows that patients with T2DM have increased circulating sclerostin levels that might contribute to the documented increased bone fragility of these patients. Of particular clinical relevance is the finding that MET and PIO have different effects on sclerostin levels and biochemical markers of bone turnover, with MET having a clearly more favorable bone profile.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by and carried out within the FP7 programme TALOS and funded by the EC (Grant Number: TALOS:Health-F2-2008-201099). The PIRAMID study was an investigator-initiated study supported by Eli Lilly, The Netherlands, which has a partnership with Takeda, the manufacturer of pioglitazone. Metformin tablets and matching placebos were kindly provided by Merck, The Netherlands.

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Received 9 December 2011

Revised version received 10 January 2012

Accepted 20 January 2012