Gender differences in sclerostin and clinical characteristics in type 1 diabetes mellitus

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Abstract

Background: Sclerostin is an osteocyte-derived inhibitor of the Wnt/ β -catenin signaling pathway, which acts as a negative regulator of bone formation. Published data on sclerostin levels in type 1 diabetes mellitus (T1DM) are few.

Objective: To evaluate gender differences in sclerostin serum levels and the association among sclerostin, bone mass, bone metabolism, and the main clinical characteristics of subjects with T1DM.

Design and methods: A total of 69 patients with T1DM (mean age, 33.7 ± 8.1 ; 49% males) were enrolled in this cross-sectional study in a clinical research center. Bone mineral density was measured by phalangeal quantitative ultrasound (QUS); bone turnover markers (urinary pyridinoline, deoxypyridinoline (D-PYR), and urine hydroxyproline (OH-PRO) to evaluate bone resorption; serum bone alkaline phosphatase and BGP to evaluate bone formation) and sclerostin were assessed.

Results: D-PYR and sclerostin were significantly higher in women when compared with men ($P=0.04$). A disease duration > 15 years was associated with higher sclerostin levels ($P=0.03$). Bone turnover markers and QUS parameters were not correlated with sclerostin. A significant negative correlation was observed among QUS parameters, BMI, and OH-PRO. Sclerostin serum levels were correlated with homocysteine ($r = -0.34$, $P=0.005$) and vitamin B12 ($r = -0.31$, $P=0.02$). Generalized linear model showed that macroangiopathy was the only predictor of sclerostin serum levels ($\beta = -11.8$, 95% CI from -21.9 to -1.7 ; $P=0.02$).

Conclusions: Our data demonstrate that women with T1DM exhibit higher sclerostin levels than men and that circulating sclerostin is not associated with bone turnover markers and phalangeal QUS measurements. Macroangiopathy was associated with sclerostin levels.

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Introduction

Several studies have demonstrated a strong association between diabetes mellitus (DM) and bone impairment, which lead to an increasing fracture risk in subjects with type 2 DM (T2DM), but mainly in subjects with T1DM (1). A reduced bone formation as a consequence of reduced osteoblast activity has been identified as the main pathogenetic effect or for low bone mineral density (BMD) in subjects with T1DM (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39).

Recently, the Wnt signaling pathway has been recognized as a major trigger of osteoblastic proliferation and differentiation, leading to bone formation (8). Moreover, WNT proteins are known to play a pivotal role in development and homeostasis processes by regulating cell differentiation, proliferation, and apoptosis in different tissues comprehensive of bone (4, 5, 6). Emerging evidences are showing that the Wnt signaling pathway is involved in cardiovascular pathophysiology (7, 8). Vascular smooth muscle cell (VSMC) proliferation causes

intimal thickening in atherosclerosis, and Wnt/ β -catenin signaling is a regulator of VSMC behavior (9). WNT proteins have been detected in murine and human atherosclerotic lesions suggesting a role for Wnt signaling in atherosclerosis (10).

The Wnt signaling pathway is modulated by a number of factors that include, among others, sclerostin, which has been identified as a main regulator of bone formation. By inhibiting the Wnt signaling pathway, sclerostin reduces osteoblast proliferation and function, thereby decreasing bone formation. Encoded by the gene *SOST*, sclerostin is expressed almost exclusively in the osteocytes that represent 95% of all bone cells. The role of sclerostin in bone homeostasis is underlined by two rare skeletal human diseases, i.e. van Buchem disease and sclerosteosis, characterized by high bone mass and impaired sclerostin production (11). However, experimental and clinical evidences demonstrated the expression of sclerostin and WNT molecules also in calcifying vascular tissues (12, 13). Sclerostin has been localized in the subendothelial layer of aortic intima in human aortic samples of atherosclerosis (14), giving new insights into the association between atherosclerosis and osteoporosis (15), and supporting the hypothesis that osteoporosis and cardiovascular diseases could be, at least in part, the pleiotropic consequences of impaired Wnt signaling.

Examining a cohort of patients with T1DM and T2DM, Gennari *et al.* (16) reported that subjects with T2DM but not those with T1DM showed higher sclerostin serum levels when compared with healthy matched controls. Differently, Neumann *et al.* (17) have recently reported that subjects with T1DM exhibited higher sclerostin concentrations in comparison with controls.

To our knowledge, a gender difference in sclerostin levels and the possible association between circulating sclerostin concentrations and patient clinical characteristics, including diabetes-related complications, have not yet been reported in subjects with T1DM.

The aims of this study were to evaluate the gender differences in bone mass and metabolism and to analyze the relationship between sclerostin and the main clinical characteristics in a wide cohort of patients with T1DM.

Methods

Study design and participants

Our cross-sectional study included a group of Caucasian subjects with T1DM, consecutively referred to the Diabetic

Outpatients Clinic of the Department of Clinical and Experimental Medicine, University Hospital of Messina (Messina, Italy) from January 2010 to May 2011. Exclusion criteria were as follows: age <18 years, pregnancy, current or previous use of oral contraceptives, corticosteroids, or other medications known to affect bone and mineral metabolism (including calcium and vitamin D; with the only exception of levothyroxine for the treatment of hypothyroidism), chronic renal failure, celiac disease, or other diseases affecting bone health. Owing to the known increase in sclerostin serum levels caused by estrogen deprivation in postmenopausal women, four postmenopausal women were excluded from the analysis in this study. All the recruited subjects did not show any clinical signs of hypogonadism. The study was conducted with the approval of the Local Ethics Committee and conformed to the Declaration of Helsinki. Written informed consent was obtained from all subjects before participating in this study.

Clinical evaluation

Height and weight were measured at baseline according to standard procedures and the BMI was calculated as weight divided by the square of height in meters. For each patient, information about the presence of diabetic complications recorded in clinical charts was evaluated. In particular, macrovascular disease was defined as a history of a cardiovascular event and/or ischemic electrocardiogram abnormalities at rest or in a stress test, or the presence of plaques detected by ultrasonographic examination of the carotid arteries or the peripheral arterial vessels or as the presence of an intima media of thickness >1.5 mm (18); sensory-motor neuropathy was diagnosed by the vibration perception test, the monofilament pressure sensation test, or electromyography; nephropathy was defined as an increased urinary albumin excretion (albuminuria) diagnosed if urinary albumin concentration was >30 mg/l, or if urinary albumin excretion rate was >20 μ g/min, or if urinary albumin-to-creatinine ratio was >2.5 mg/mmol in men and 3.5 mg/mmol in women; and retinopathy was detected by high-quality fundus photographs (19). All subjects were free from diseases or comorbidities impairing normal daily activity. Further information regarding duration of disease, total daily insulin requirements, and insulin therapeutic schemes (continuous subcutaneous insulin infusion (CSII) system or multiple daily injections) was collected. Participants reported alcohol use, smoking status, and level of physical activity in a specific health questionnaire.

Biochemical measurements

Biochemical evaluation was conducted on serum and 2-h morning urine samples obtained after an overnight fast. Serum and urinary calcium, phosphorus, serum and urinary creatinine, alkaline phosphatase (ALP), bone ALP (B-ALP), as a marker of bone formation, and HbA1c were measured using standard laboratory techniques; PTH, folic acid, vitamin B12, 25-hydroxyvitamin D and urine hydroxyproline (OH-PRO), pyridinoline (PYR), and deoxy-pyridinoline (D-PYR), as markers of bone resorption, were determined by HPLC; and osteocalcin (BGP), a marker of bone formation, and homocysteine levels were measured by immunoenzymatic assay. Serum sclerostin was measured using a commercially available ELISA Kit (Biomedica, Vienna, Austria) according to the manufacturer's instructions with intra- and inter-assay coefficient of variation (CV) values of 5 and 6% respectively.

Bone mass measurements

To assess bone mass, we performed quantitative ultrasound (QUS) measurements at the proximal phalangeal metaphysis of the last four fingers of the nondominant hand using a DBM Sonic Bone Profiler (Igea, Carpi, Italy), as described previously (20). Briefly, two probes mounted on a precision caliper, one acting as generator of the signal and the other one as receiver, were positioned on the lateral and medial surfaces of each finger. Standard ultrasound gel was used to couple probes to the skin. A Plexiglass phantom served to calibrate the device as recommended by the manufacturer. The following parameters were measured: amplitude-dependent speed of sound (AD-SoS), bone transmission time (BTT), fast-wave amplitude (FWA), signal dynamic (SDy), and ultrasound bone profile index (UBPI) calculated automatically ($UBPI = -(0.0018 \times SDy - 0.0560 \times FWA - 0.0560 - 1.1467 \times BTT + 3.0300)$). In accordance with the reference data from the manufacturer, T- and Z-score values were also evaluated. The same trained operator, who was blinded to clinical parameters, performed the QUS measurements. The CV value, determined by repeated measurements in a subgroup of 25 subjects, was of 0.9%.

Statistical analysis

Data were expressed as means $\pm$ s.d. for continuous variables and percentages for categorical variables. Characteristics of the study population were categorized by gender. The Kolmogorov–Smirnov test was used to test

the normality of distribution of continuous variables. For continuous variables, mean values between two groups were compared by unpaired Student's *t*-test for normally distributed variables and the Mann–Whitney *U* test for skewed variables. The χ^2 -test was used to compare categorical variables between groups. Pearson's correlation coefficient was employed to test correlations among sclerostin, bone turnover markers, and QUS parameters. To determine the independent variables correlated with sclerostin (dependent variable), a generalized linear model was performed. The following variables were included in the model: gender, age, BMI, duration of disease, HbA1c, macrovascular disease, retinopathy, and neuropathy. A *P* value of <0.05 was considered as statistically significant. Statistical analysis was performed using the Statistical Package for Social Science Software (SPSS Statistics, 17.0).

Results

Overall, 69 subjects with T1DM were recruited and their clinical data are shown in Table 1. All participants were considered together and divided into two groups according to gender. When considering T1DM subjects by gender, there were no statistical differences in age, BMI, duration of disease, smoking, HbA1c, and prevalence of retinopathy and neuropathy. A statistical difference between groups was shown regarding insulin treatment schemes, women using more CSII devices, and in macroangiopathy, men in whom this condition occurs more frequently.

Food energy, estimated mean calcium intake, alcohol consumption, physical activity, and insulin doses were not significantly different in men in comparison with women (data not shown).

QUS parameters were not significantly different between men and women (Table 2). No significant difference by gender was observed regarding bone formation markers (B-ALP and BGP); differently, urinary level of D-PYR, a marker of bone resorption, was significantly higher in women revealing a slight increased turnover rate in comparison with men.

Sclerostin serum levels were higher in women, even after adjustment for BMI and age ($P=0.04$; Fig. 1). However, sclerostin was not significantly different in all T1DM subjects in comparison with a group of ten age-matched healthy controls (13.33 ± 11.14 vs 14.21 ± 13.15 respectively; $P>0.05$; Table 1). When considering the duration of disease, subjects of both genders showed higher serum sclerostin levels in case of a disease duration greater than the median value of 15 years, when compared

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Table 1 Clinical characteristics of the study population according to gender.

	T1DM subjects				Controls			
	Overall (n=69)	Males (n=34)	Females (n=35)	P	Overall (n=10)	Males (n=5)	Females (n=5)	P
Age (years)	33.7±8.1	32.8±9.3	34.5±6.9	0.39	32.8±8.0	32.4±8.7	32.2±8.3	0.88
BMI (kg/m ²)	24.5±3.4	24.8±3.6	24.3±3.2	0.52	24.1±2.2	24.2±2.1	24.0±2.1	0.94
Smokers (%)	36.2	29.4	42.9	0.18	30	40	20	0.80
Duration of disease (years)	15.4±8.8	14.9±9.3	15.9±8.4	0.64				
Patients with CSII (%)	30.4	17.6	42.9	0.02				
HbA1c (proportion of total Hb)	0.08±0.01	0.08±0.02	0.08±0.1	0.90				
Diabetic complications (%)								
Macrovascular disease	14.5	23.5	5.7	0.04				
Nephropathy	7.2	5.9	8.6	0.67				
Retinopathy	37.7	44.1	31.4	0.20				
Neuropathy	13.0	5.9	20.0	0.08				

with a lower duration of disease (16.5 ± 11.3 vs 10.3 ± 10.3 respectively; $P=0.03$), even after adjusting for age and BMI; this difference disappeared when adjusting even for impairment of renal function ($P=0.56$).

Sclerostin levels differed, but not significantly, in patients with macroangiopathy (7.9 ± 7.0 vs 13.8 ± 11.4 for macroangiopathy yes vs macroangiopathy no, $P=0.18$; Fig. 2), retinopathy (15.6 ± 12.9 vs 11.7 ± 10.0 , for retinopathy yes vs retinopathy no, $P=0.34$), and neuropathy (21.3 ± 16.3 vs 11.8 ± 9.6 for neuropathy yes vs neuropathy no, $P=0.12$). Men with T1DM showed significantly higher homocysteine levels albeit no differences in folate and vitamin B12 levels were detected in comparison with women (Table 2). A significant negative correlation was observed between the BMI and UBPI ($r=-0.32$, $P=0.008$), AD-SoS ($r=-0.52$, $P<0.0001$), T -score ($r=-0.52$, $P<0.0001$), and Z -score ($r=-0.44$, $P<0.0001$).

QUS parameters were also correlated with OH-PRO ($r=-0.26$, $P=0.03$; $r=-0.32$, $P=0.009$; $r=-0.27$, $P=0.003$; and $r=-0.40$, $P=0.001$ for AD-SoS, UBPI, T -score, and Z -score respectively) and PYR ($r=-0.34$, $P=0.006$ for AD-SoS, UBPI, and Z -score; $r=-0.38$, $P=0.002$ for T -score). Sclerostin serum levels were correlated with vitamin B12 ($r=-0.31$, $P=0.02$) and homocysteine ($r=-0.34$, $P=0.005$) levels but not with bone turnover markers or QUS measurements. A generalized linear model showed that macroangiopathy was the only predictor of sclerostin serum levels in the whole study population ($\beta=-11.8$, 95% CI from -21.9 to -1.7 ; $P=0.02$).

Discussion

Our study investigated bone mass and metabolism in subjects affected by T1DM and explored the relationship

Table 2 Laboratory data and phalangeal quantitative ultrasound measurements according to gender.

	Overall (n=69)	Males (n=34)	Females (n=35)	P
Calcium (mmol/l)	2.23±0.1	2.25±0.1	2.2±0.08	0.006
Phosphorus (mmol/l)	1.23±0.19	1.20±0.19	1.23±0.19	0.78
Serum creatinine (mmol/l)	61.88±17.68	70.72±17.68	53.04±8.84	<0.0001
25-Hydroxyvitamin D (nmol/l)	66.14±26.71	60.90±22.46	71.39±29.95	0.11
PTH (ng/l)	16.6±5.8	16.5±6.3	16.6±5.4	0.94
Osteocalcin (µg/l)	6.2±5.9	6.5±4.6	6.0±6.9	0.71
Bone alkaline phosphatase (µkat/l)	0.63±0.16	0.60±0.16	0.67±0.15	0.08
Hydroxyproline (pmol/µmol urinary creatinine)	30.1±15.8	29.3±19	30.9±12.2	0.68
Pyridinoline (pmol/µmol urinary creatinine)	59.0±32.1	52.0±29.2	65.7±33.8	0.08
Deoxypyridinoline (pmol/µmol urinary creatinine)	15.4±7.7	13.1±4.9	17.7±9.1	0.01
Urinary calcium/urinary creatinine ratio	0.09±0.08	0.10±0.09	0.08±0.06	0.35
Sclerostin (pmol/l)	13.1±11.1	9.6±7.9	15.6±12.5	0.04
Homocysteine (µmol/l)	11.2±5.1	13.6±6.1	8.8±1.6	<0.0001
Folic acid (nmol/l)	14.5±17.45	15.41±23.79	13.82±6.12	0.72
Vitamin B12 (pmol/l)	468.72±331.49	489.97±422.1	446.81±204.96	0.60
AD-SoS (m/s)	2002.3±102	2009.7±113.6	1994.6±89.5	0.55
UBPI (U)	0.54±0.19	0.56±0.22	0.53±0.17	0.51
T -score (s.d.)	-1.73±1.45	-1.63±1.62	-1.85±1.28	0.54
Z -score (s.d.)	-1.71±1.6	-1.68±1.89	-1.75±1.26	0.86

Figure 1

Serum sclerostin levels according to gender in T1DM and healthy control subjects. Data are expressed as mean $\pm$ s.d.

between sclerostin and the main clinical features of patients with T1DM. Sclerostin is an osteocyte-derived glycoprotein, which plays a key role in the regulation of the canonical Wnt signaling pathway. Sclerostin is critical for osteoblast proliferation and activity, not only leading to inhibition of bone formation, but also encouraging bone resorption (21). Humans with genetic deficiencies of sclerostin and mice with knockout of the *Sost* gene have high bone mass and increased bone strength. Sclerostin works by binding to the LDL receptor-related proteins 5 and 6 (LRP5 and LRP6), leading to translocation of β -catenin into the nucleus and to regulation of targeted gene expression (4, 5, 6). To date, few data have been published on sclerostin in diabetes and only two studies have reported sclerostin serum levels in subjects with T1DM analyzing their relationship with bone turnover markers and bone mass (16, 17). However, these cohorts were heterogeneous for a number of participants, age, and duration of disease ranges when compared with our study population. Our first interesting and furthermore innovative result was that circulating sclerostin levels were significantly increased in women when compared with men with T1DM. With few exceptions (22), most of the existing data in the literature (23, 24) showed higher sclerostin levels in men to an extent diametrically opposed with our findings; furthermore, they are based on studies involving subjects affected by T2DM (30). Even Gennari *et al.* (16) showed higher sclerostin levels in men but the overall cohort also included T2DM subjects and healthy controls; for this reason, their results are not generalizable

to T1DM. Otherwise, after adjusting for age, no differences in sclerostin serum levels by gender were found in subjects with T1DM in the study by Neumann *et al.* (17). In our opinion, the fact that the women we studied had higher sclerostin levels was not a coincidence, because i) we performed serum sclerostin measurements using the same method and the same commercially available kit that was also used by Gennari *et al.* and ii) the women we studied showed a higher bone turnover rate when compared with men, and we can reasonably hypothesize that sclerostin may have contributed to the increased bone resorption. Modder *et al.* (23) studying sclerostin serum levels in a population-based sample of 362 women and 318 men observed that, at any age, serum sclerostin levels were higher in men than in women. They concluded that reasons for this are unclear, except that circulating sclerostin levels might reflect total-body skeletal mass and the larger skeleton in men simply may produce and release more sclerostin into the circulation. This hypothesis can be realistic after the study conducted by Amrein *et al.* (24), in which men had significantly higher unadjusted sclerostin levels than women but levels were not significantly different after adjustment for skeletal size. Our data raise the issue of the role of sex steroids in explaining gender differences in sclerostin serum levels: both estrogen and testosterone are critical regulators of bone turnover and have been inversely associated with sclerostin levels (25, 26). Disturbances in these

Figure 2

Serum sclerostin levels in T1DM subjects with and without macroangiopathy. Data are expressed as mean $\pm$ s.d.

hormones were reported previously in T1DM (27, 28, 29). Although the recruited subjects did not exhibit any clinical sign of hypogonadism or other hormonal disorders, however, we cannot rule out biochemical hormonal abnormalities having not looked at circulating sex hormones. Sclerostin serum levels were significantly increased in women with T1DM who also showed a higher bone turnover rate when compared with men, and we hypothesized that sclerostin may have contributed to the increased bone resorption. Interestingly, we found significantly higher sclerostin serum levels in subjects with long disease duration (>15 years) when compared with subjects without long disease duration (<15 years). As sclerostin level increases with age and it has been suggested to be contributing to age-related bone loss (23), it might be supposed that increased sclerostin levels in long disease duration could play a role in explaining, at least in part, the effects of diabetes duration on bone tissue *per se*.

In our population, poor QUS values were observed in subjects with T1DM with increased bone resorption, a higher BMI, and low vitamin D levels, but we did not observe an association between bone density detected by phalangeal QUS and sclerostin levels. The latter is consistent with the data reported by Gennari *et al.* (16) using DXA, although an association between sclerostin serum levels and DXA measurements was also described in T1DM (17). Besides BMD, in previous works, sclerostin was associated with fracture risk, although heterogeneous results were reported (31, 32). In particular, sclerostin was related to vertebral fractures in T2DM (33). Data on the association between sclerostin levels and fracture risk in T1DM are still lacking.

The novelty of this research remains the link between sclerostin and macroangiopathy, which has never been reported before in T1DM. Recently, but in a population of subjects with T2DM, Morales-Santana *et al.* (30) have observed an association between circulating sclerostin and surrogate markers of atherosclerotic disease such as abnormal carotid intima-media thickness (IMT), carotid plaque, and aortic calcification. Sclerostin has been found to be involved in vascular calcification (VC) (13, 14), and higher sclerostin levels were detected in postmenopausal women with a higher pulse wave velocity (34). Lower sclerostin serum levels were identified as independent determinants of VC in patients with chronic kidney disease not yet on dialysis, thus it was suggested that circulating sclerostin may be a protective factor for the progression of VC (35). Consistent with this, higher sclerostin levels have been associated with a reduced mortality in hemodialysis patients (36).

As known, cardiovascular disease as a result of macrovascular atherosclerotic progression is the major cause of mortality among patients with diabetes, and atherosclerosis had already been observed in childhood with T1DM. Arterial wall is the second most extensively calcified structure in the human body, and VC is a complex process involving cellular phenotypic switch from VSMCs to mineralizing osteoblast-like cells. These phenotypic changes permit arterial VSMC migration into the intima where these cells are able to express several osteogenic genes such as the osteoblastic transcription factor, *Cbfa1/Runx2*, bone morphogenetic protein 2 (BMP2), and *Msx2* (a promoter of early osteoblast development) in addition to extracellular matrix and matrix-degrading metalloproteinases (12, 13, 37). The prevalence of coronary atherosclerosis is similar between patients with T1DM and those with T2DM, with a relatively higher proportion of noncalcified plaques observed in patients with T2DM in comparison with those with T1DM (38). As T2DM was reported previously to show higher sclerostin levels than T1DM (16), it is reasonable to speculate that sclerostin could act as an inhibitor of VC in T2DM. At vascular level, β -catenin activity, under control by N-cadherin and reactive oxygen species (ROS), drives VSMC proliferation and neointima formation, thus sclerostin might attenuate the canonical Wnt signaling pathway stimulated by peroxide via an ROS-sensitive nucleoredoxin-disheveled relay (39). Therefore, it is likely that macroangiopathy in T1DM could be linked to lower sclerostin serum levels. In our study, women exhibited higher sclerostin levels and a lower prevalence of macroangiopathy when compared with men who, on the other hand, showed higher homocysteine levels. Sclerostin was negatively associated with homocysteine in the whole population of T1DM, in contrast to what has recently been observed in T2DM (30). These data may account for an increased cardiovascular risk in men in comparison with women with T1DM. In addition, our results seem to be in line with a recent report showing that, in T2DM postmenopausal women, sclerostin was negatively associated with IMT, and was an independent predictor of IMT in multivariate linear regression analysis (40). Taken together with our findings, these evidences suggest that sclerostin may be involved in the delay of atherosclerosis progression.

In conclusion, our study is the first one that has investigated the relationship between serum sclerostin levels and the main clinical characteristics in subjects with T1DM according to gender, suggesting that macroangiopathy was predictive of circulating sclerostin. We must

recognize, however, some limitations of this research: the relatively small sample size to account for more specific associations between sclerostin and T1DM-related complications and the lack of DXA measurements of bone mass, although we used a technique that was found to correlate with DXA. Moreover, this being a cross-sectional study no causal inferences can be made; in fact, the cross-sectional design does not allow establishing a cause–effect relationship between sclerostin and macroangiopathy. Future longitudinal studies are required to investigate the role of sclerostin in vascular pathology. Observing changes in sclerostin levels over time and correlating these changes with those observed in metabolic and vascular morphological parameters could provide a contribution far greater than our results. Addressing this issue is critical, as inhibition of sclerostin, via sclerostin-neutralizing antibodies, is an upcoming intriguing anabolic treatment for osteoporosis, but the potential cardiovascular effects should be carefully evaluated even in subjects with T1DM.

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.

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