The differences in the relationship between obstructive sleep apnea severity and trabecular bone score in men and women with type 2 diabetes

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ABSTRACT

Aims: Type 2 diabetes mellitus (T2DM) and obstructive sleep apnea (OSA) may adversely affect bone. Gender is a well-established factor influencing bone health. We investigated the impact of OSA on bone mineral density (BMD) and trabecular bone score (TBS) in T2DM.
Methods: Eighty-one T2DM patients [33 men and 48 women] participated. OSA was diagnosed using an overnight monitor, with its severity assessed by an apnea hypopnia index (pAHI). The measurements of hypoxia, including the percentage of total sleep time in which oxygen saturation remains below 90% (pT90), the oxygen desaturation index (pODI) and minimum O_2 (min O_2), were reported. Lumbar spine (L1-4) and femoral neck (FN) BMD were measured using dual-energy X-ray absorptiometry (DXA). TBS was computed from DXA images.
Results: Sixty-five patients (80.2%) had OSA. pAHI, pT90, pODI and min O_2 were not correlated to L1-4 BMD, FN BMD or TBS in all participants by multiple regression analyses adjusting for age, gender and BMI. However, an interaction between gender and pAHI, and gender and pODI were significantly associated with TBS (b = 0.003, p = 0.034 and b = 0.004, p = 0.046, respectively). We therefore reassessed an association between pAHI or pODI and TBS separately between men and women. After adjusting for age and BMI, more severe OSA (higher pAHI) and higher pODI significantly associated with lower TBS (b = −0.002, p = 0.034 and b = −0.003, p = 0.021, respectively) in men. On the other hand, higher pAHI non-significantly associated with better trabecular microarchitecture as indicated by higher TBS (b = 0.002, p = 0.059) in women. When considered only postmenopausal (n = 33), higher pAHI and higher pODI were significantly associated with higher TBS (b = 0.004, p = 0.003 and b = 0.004, p = 0.008, respectively).
Conclusions: In T2DM patients, there is a complex interrelationship among OSA severity, gender and TBS. More severe OSA predicted lower TBS in men, but predicted higher TBS in postmenopausal women.

Introduction

Type 2 diabetes mellitus (T2DM) is the most common metabolic disorder worldwide. Long disease duration and poor glycemic control are risk factors for complications, including osteoporotic fractures [1].

Alterations in bone quality and strength due to hyperglycemia, glycemic-lowering agents (e.g., thiazolidinedione and insulin) and increased fall risk contribute to bone disease in T2DM [2]. While bone mineral density (BMD) is the only conventional bone quantity assessment, it has limitations in predicting fracture risk in patients with

diabetes. Despite having higher BMD, patients with T2DM had a higher risk for osteoporotic fracture than those without T2DM, possibly due to poor bone quality [3].

Recently, trabecular bone score (TBS) has been used to capture information related to trabecular microarchitecture (including trabecular number, separation and connectivity density). TBS can predict fracture risk in osteoporosis independent of BMD [4,5]. Data suggested that while areal BMD (aBMD) tended to be higher in participants with T2DM, their TBS was found to be lower than those without T2DM [5–8]. Therefore, TBS has been recognized as a predictor of incident fracture in T2DM in addition to aBMD [9].

Obstructive sleep apnea (OSA) is a common sleep disorder and recognized as a risk factor for insulin resistance and T2DM, independent of the degree of obesity [10]. OSA is characterized by repetitive episodes of upper airway closure or partial collapse during sleep, resulting in intermittent hypoxia. OSA is highly prevalent in patients with T2DM in both Western [11] and Asian countries [12,13]. Emerging evidence suggested that multiple hormonal and inflammatory derangements related to OSA may adversely affect bone [14]. To date, studies investigated the impact of OSA on BMD were mostly conducted in non-diabetic populations, and the results are conflicting. It has been reported that OSA could impair [15–17], stimulate [18,19] or not affect [20] bone metabolism. Controversial results could be explained by the differences in study population including age, gender, menopausal status, the presence of disease known to affect bone health including T2DM.

To date, the effects of OSA on bone mass and trabecular microarchitecture exclusively in T2DM population have not been studied. In addition, gender, a well-established factor influencing on bone health, was less likely to be considered in the studies exploring the effects of OSA on bone health. In this study, we investigated the impact of OSA on BMD/TBS in T2DM according to gender.

Subjects and methods

Men or women with T2DM who were being followed in the endocrinology clinic at the Faculty of Medicine Ramathibodi Hospital, Mahidol University, were invited to participate. Exclusion criteria included having previously diagnosed OSA, being currently pregnant, or performing shift work. Additionally, patients with a history of congestive heart failure or low ejection fraction, chronic obstructive pulmonary disease, end-stage renal disease, severe chronic liver disease such as cirrhosis, stroke, permanent pacemaker placement, or use of certain medications (opioids/narcotics, alpha-adrenergic blockers, clonidine, methyldopa, nitroglycerin) were also excluded in order to obtain valid results from the OSA diagnostic method utilized (see below). Further exclusion criteria were diseases that affect bone metabolism, such as hyperthyroidism, primary hyperparathyroidism, Cushing syndrome, hypogonadism and malignancy. Patients on glucocorticoids, parathyroid hormone, bisphosphonates, strontium ranelate or hormone replacement therapy were also excluded. All participants gave written informed consent. The protocol was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand (ID 02-57-22).

Assessment of diabetes history and glycemic control

After obtaining informed consent, research personnel interviewed participants about their diabetes history and management. Height, age, current medications and most recent hemoglobin A1c (HbA1c) values were extracted from medical records. The HbA1c assay at Ramathibodi Hospital has been certified by the NGSP (National Glycohemoglobin Standardization Program). Weight was measured with a calibrated electronic scale (seca 767; Hamburg, Germany), having an accuracy of 0.01 kg. Height, previously assessed at the outpatient clinic using a

wall-mounted stadiometer (with standard method and barefoot), was extracted from medical record. Body mass index (BMI) was then calculated as weight (kg)/height^2 (m^2). Exercise data, including aerobic and strengthening exercise, were derived from questionnaire response. The frequency of aerobic exercise was reported in three categories; no exercise, 1–2 times/week and ≥ 3 times/week. The number of subjects who performed weight-bearing exercise and strengthening exercise were additionally described. Detail of smoking status (currently smoking vs. no) and alcohol use (defined as currently consuming ≥ 2 units/day in women or ≥ 3 units/day in men) [21] were obtained from a questionnaire.

Assessment of OSA

OSA was diagnosed using an FDA-approved portable diagnostic device, WatchPAT 200 (Itamar Medical, Caesarea, Israel), which has been validated against polysomnography in populations with and without diabetes [22,23]. This non-invasive device is shaped similar to a large watch, and is worn on the non-dominant wrist immediately before bedtime and removed upon awakening in the morning. The device has two probes connecting to the participant's fingers to measure changes in peripheral arterial tone (PAT) and oxygen saturation, along with built-in actigraphy to measure sleep time. The severity of OSA is assessed by PAT apnea–hypopnea index (pAHI), which is automatically generated by the software using changes in the peripheral arterial tonometry. OSA is considered present if pAHI ≥ 5. OSA is considered mild, moderate and severe if pAHI $\geq 5 - < 15$, ≥ 15–30, and > 30, respectively. Other measurements of hypoxia were assessed. These included the percentage of total sleep time in which oxygen saturation remains below 90% (pT90), the oxygen desaturation index (pODI), which is the average number of times per hour of sleep time that the oxygen saturation drops by 3% or more, and minimum O$_2$ (min O$_2$), which is the lowest oxygen saturation value. Because this device relies on changes in peripheral arterial tone, the use of certain medications (opioids/narcotics, alpha-adrenergic blockers, clonidine, methyldopa, nitroglycerin) was not allowed. In addition, it cannot differentiate obstructive from central apnea events. Therefore, we also excluded patients with certain conditions which may pose a higher risk for central apnea. These included patients with a history of congestive heart failure or low ejection fraction, chronic obstructive pulmonary disease, end-stage renal disease, severe chronic liver disease such as cirrhosis, stroke, and permanent pacemaker placement.

BMD and TBS measurement

BMD assessment

As previously reported [24], each subject changed into light clothing before undergoing BMD assessment by DXA at the lumbar spine (L1-L4 vertebrae) and hip [femoral neck (FN)]. Using fast-array mode, all measurement procedures were performed according to the International Society for Clinical Densitometry (ISCD) recommendations [25] by ISCD-certified densitometry technologists using the same Hologic Discovery DXA scanner on all subjects (Hologic, Bedford, MA). The BMD root mean square (RMS) coefficient of variation (CV) and RMS standard deviation (SD) were, respectively: 0.69% and 0.006 g/cm^2 at the lumbar spine; and 1.68% and 0.012 g/cm^2 at the FN. One participant (women) received vertebral fixation. Therefore, BMD at L1-4 and TBS were obtained from only 80 subjects.

TBS assessment

Using TBS iNsight® software version 2.1 (Medimaps, Geneva, Switzerland) on the same regions of interest as those used for lumbar spine BMD, TBS was calculated as the mean value of the individual measurements for each vertebra and for every combination of ROI from L1 through L4 vertebrae. The RMS SD and RMS CV of TBS were 0.026 and 2.05%, respectively.

Statistical analysis

Continuous variables which were normally distributed were expressed as mean ± SD, and those without a normal distribution were expressed as median (interquartile range, IQR). Categorical data were expressed as frequencies/percentages. Comparisons the difference between men and women were performed by independent-Samples *T* Test, Mann-Whitney *U* test, Pearson's chi-squared test and Fisher's exact test, as appropriate.

Univariate linear regression analyses were used to explore associations between clinical characteristics, sleep parameters (pAHI, pT90, pODI, min O_2) and BMD/TBS L1–4. Multiple linear regression was used to determine the independent predictor of BMD/TBS. Sleep parameters and factors that could influence bone or factors with $p < 0.1$ in the univariate analysis (i.e., age, gender, BMI) and were entered in the first model. To test whether an interaction between gender and sleep parameters could predict BMD/TBS, an interaction between gender and each sleep parameters was further added to the second model. Subjects were then classified in to two group according to gender, and the association between BMD/TBS and age, BMI and sleep parameters were reassessed by multiple linear regression analysis. The correlation between pAHI or pODI and TBS in men and women were demonstrated in the figure and used Pearson correlation. Analyses were performed using SPSS statistical software package, version 18.0 (SPSS, Chicago IL, USA).

Results

Eighty-one participants (33 men and 48 women) with mean age of 54.7 years, mean HbA1c of 7.6% and median duration of diabetes of 8 years were included in this study (Table 1). The mean BMI was 28.3 kg/m². Sixty-five participants (80.2%) were diagnosed with OSA.

Table 2
Simple regression analysis between factors and L1-4 BMD, FN BMD and TBS.

Characteristics	L1-4 BMD[a]		FN BMD		TBS[a]	
	b	p value	b	p value	b	p value
Age	−0.004	0.006	−0.005	< 0.001	−0.002	0.072
DM duration	0.001	0.749	−0.003	0.100	0	0.866
Gender	−0.052	0.153	−0.036	0.235	−0.048	0.029
Insulin use	0.050	0.206	0.012	0.707	0.016	0.500
BMI	0.008	0.021	0.011	< 0.001	−0.005	0.024
HbA1c	0.017	0.186	0.013	0.210	0.002	0.833
Aerobic exercise	0.050	0.186	0.021	0.501	−0.028	0.216
Strengthening exercise	0.040	0.808	0.052	0.706	−0.114	0.248
Current smoking	−0.069	0.407	0.081	0.244	−0.081	0.106
pAHI	0.001	0.327	0.000	0.655	0.000	0.645
pT90	0.002	0.531	0.000	0.782	−0.003	0.072
pODI	0.001	0.596	0.000	0.973	0.000	0.361
Min O_2	−0.002	0.504	−0.001	0.601	0.002	0.232

L1–4 BMD, L1–4 bone mineral density; FN BMD, femoral neck BMD; TBS, trabecular bone score L1–4; BMI, body mass index; p, peripheral arterial tone (PAT); pAHI, PAT-derived apnea–hypopnea index; pT90, PAT-derived T90; pODI, PAT-derived oxygen desaturation index; min O_2, minimum O_2.
Gender, men = 0, women = 1; insulin use, no = 0, yes = 1; aerobic exercise, no = 0, yes = 1; strengthening exercise, no = 0, yes = 1; current smoking, no = 0, yes = 1.
    [a] $n = 80$.

Among those with OSA, 34 (42%), 21 (25.9%) and 10 (12.3%) had mild, moderate and severe OSA, respectively, with an overall median pAHI of 10.7. There were no differences in age, insulin use, BMI, HbA1c, strengthening exercise, current smoking and current alcohol use between genders. Men had longer duration of diabetes and higher

Table 1
Descriptive characteristics of the participants.

	Total n = 81	Men n = 33	Women n = 48	p value
Clinical parameters				
Age (yrs)	54.7 ± 11.7	54.3 ± 12.0	55 ± 11.6	0.794
DM duration (yrs)	8 (3.5–15.5)	10 (5.5–19.5)	6 (/2–14.5)	0.044
Insulin use [n, (%)]	24 (29.6)	13 (39.4)	11 (22.9)	0.111
BMI (kg/m²)	28.3 ± 5.0	27.2 ± 5.3	29.1 ± 4.6	0.080
HbA1c (%)	7.6 ± 1.4	7.6 ± 1.4	7.5 ± 1.5	0.849
Exercise				
1) Aerobic exercise				0.016
Frequency [n,(%)]				
0 time/week	52 (64.2)	18 (54.5)	34 (70.8)	
1–2 times/week	5 (6.2)	5 (15.2)	0	
≥3 times/week	24 (29.6)	10 (30.3)	14 (29.2)	
Weight bearing exercise [n,(%)]	25 (30.9)	14 (42.4)	11 (22.9)	
2) Strengthening exercise [n,(%)]	1 (1.2)	0	1 (2.1)	1.000
Currently smoking [n, (%)]	4 (4.9)	3 (9.1)	1 (2.1)	0.299
Current alcohol use [n, (%)]	0	0	0	NA
Sleep parameters				
pAHI	10.7 (6.3–22.6)	14.4 (8.1–27.9)	9.8 (4.8–16)	0.032
pT90	0.2 (0–1.2)	0.6 (0–1.8)	0.1 (0–1.0)	0.115
pODI	6.2 (2.1–14)	10.1 (3.9–18.9)	5.3 (1.5–10.4)	0.025
min O_2	84.9 ± 7.3	83.3 ± 6.8	85.9 ± 7.6	0.115
BMD indicators				
L1–4 BMD (g/cm²)	1.015 ± 0.160[a]	1.046 ± 0.165	0.993 ± 0.156[b]	0.152
FN BMD (g/cm²)	0.744 ± 0.135	0.766 ± 0.130	0.729 ± 0.137	0.235
TBS	1.305 ± 0.097[a]	1.333 ± 0.086	1.284 ± 0.101[b]	0.029

Data are expressed as mean ± SD or median (IQR) unless otherwise noted.
DM, diabetes; BMI, body mass index; HbA1c, hemoglobin A1c; p, peripheral arterial tone (PAT); pAHI, PAT-derived apnea–hypopnea index; pT90, PAT-derived T90; pODI, PAT-derived oxygen desaturation index; min O_2, minimum O_2; L1–4 BMD, L1–4 bone mineral density; FN BMD, femoral neck BMD; TBS, trabecular bone score L1–4.
Current smoking, currently smoking vs. no; current alcohol use, currently consuming ≥2 units/day in women or ≥3 units/day in men vs. no.
    [a] $n = 80$.
    [b] $n = 47$.

frequency of aerobic exercise than women. As expected, men had more severe OSA (higher pAHI) and higher pODI. However, there were no significant differences in pT90 and min O_2 between genders. In addition, men had higher TBS than women. There were no significant differences in L1-4 and FN BMD between genders. (Table 1).

Associations between clinical characteristics, sleep parameters and BMD indicators

Simple linear regression analyses were performed to test association between BMD indicators, clinical characteristics (age, BMI, DM duration, HbA1c and insulin use, exercise, smoking) and sleep parameters (pAHI, pT90, pODI, min O_2) (Table 2).

For associations with L1-4 BMD; younger age (b = −0.004, p = 0.006) and higher BMI (b = 0.008, p = 0.021) were associated with higher L1-4 BMD. All sleep parameters were not associated with L1-4 BMD.

For associations with FN BMD; younger age (b = −0.005, p < 0.001) and higher BMI (b = 0.011, p < 0.001) were associated with higher FN BMD. All sleep parameters were not associated with FN BMD.

For associations with TBS: younger age (b = −0.002, p = 0.072) was non-significantly associated with TBS. Male and lower BMI were associated with higher TBS. Similar to BMD, pAHI, pODI and min O_2 were not associated with TBS. However, lower pT90 (b = −0.003, p = 0.072) was non-significantly associated with TBS.

Multiple regression analysis with L1-4 BMD, FN BMD and TBS as an outcome

We performed multiple regression analysis to determine an independent association between sleep parameters and BMD/TBS. Age, gender, BMI and each sleep parameters, including pAHI, pT90, pODI, min O_2, were included in the first model. In the second model, an interaction between gender and each sleep parameter was further added.

After adjusting for age, gender and BMI, pAHI was not associated with L1-4 BMD, FN BMD and TBS (Table 3). Similar association was revealed after adding an interaction between gender and pAHI to the second model of L1-4 BMD and FN BMD. However, after adding an interaction between gender and pAHI to the second model of TBS, age (b = −0.003, p = 0.003), gender (b = −0.081, p = 0.002), BMI (b = −0.008, p = 0.002) and an interaction between gender and pAHI (b = 0.003, p = 0.034) were significantly associated with TBS (Table 3).

pT90 was not associated with L1-4 BMD, FN BMD and TBS after adjusting for age, gender and BMI. After adding an interaction between gender and pT90 to the second model, there were no association between an interaction between gender and pT90 with L1-4 BMD (b = 0.003, p = 0.683), FN BMD (b = −0.002, p = 0.673) and TBS

(b = 0.004, p = 0.290).

In the first model, pODI was not associated with L1-4 BMD, FN BMD and TBS (Table 4). Similar associations were revealed after adding an interaction between gender and pODI to the second model of L1-4 BMD and FN BMD. Of note, after adding an interaction between gender and pODI to the second model of TBS, age (b = −0.003, p = 0.004), gender (b = −0.072, p = 0.013), BMI (b = −0.007, p = 0.003), pODI (b = −0.005, p = 0.049), and an interaction between gender and pODI (b = 0.004, p = 0.046) were significantly associated with TBS (Table 4).

Similar to pT90, min O_2 were not associated with L1-4 BMD, FN BMD and TBS after adjusting for age, gender and BMI. In addition, an interaction between gender and min O_2 were not associated with L1-4 BMD (b = −0.003, p = 0.524), FN BMD (b = −0.001, p = 0.777), and TBS (b = 0.000, p = 0.987).

Multiple regression analysis with TBS as an outcome in subjects stratified by gender

Because of the potential interaction between gender and pAHI and gender and pODI, data were reanalyzed according to gender (Table 5). Firstly, we investigated the association between TBS and pAHI. In men, after adjusting for age and BMI, more severe OSA (higher pAHI) significantly associated with lower TBS (b = −0.002, p = 0.034). On the other hand, in women, higher pAHI non-significantly correlated with better trabecular microarchitecture as indicated by higher TBS (b = 0.002, p = 0.059) independent of age and BMI. However, when considered only postmenopausal women (n = 33), higher pAHI became significantly associated with TBS (b = 0.004, p = 0.003) after adjusting for age and BMI. pAHI, age and BMI were not associated with TBS in an analysis of premenopausal women (n = 14). Fig. 1a and 1b demonstrated an opposite direction of an association between TBS and pAHI in men (r = −0.460, p = 0.006) and postmenopausal women (r = 0.284, p = 0.109).

Secondly, the association between TBS and pODI was assessed. In men, after adjusting for age and BMI, higher pODI significantly associated with lower TBS (b = −0.003, p = 0.021). In women, higher pODI was not correlated to TBS. However, when considered only postmenopausal women and adjusting for age and BMI, higher pODI became significantly associated with TBS (b = 0.004, p = 0.008). In premenopausal women, pODI, age and BMI were not associated with TBS. Fig. 1c and 1d demonstrated an opposite direction of an association between TBS and pODI in men (r = −0.437, p = 0.011) and postmenopausal women (r = 0.289, p = 0.103).

Discussion

To our knowledge, this is the first study exploring the association between OSA severity and bone mass and trabecular microarchitecture

Table 3
Multiple regression analysis between factors, pAHI and L1-4 BMD, FN BMD and TBS.

	L1-4 BMD[a]				FN BMD				TBS[a]			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	b	p value	b	p value	b	p value	b	p value	b	p value	b	p value
Age	−0.003	0.074	−0.003	0.098	−0.004	0.006	−0.004	0.006	−0.003	0.002	−0.003	0.003
Gender	−0.058	0.126	−0.095	0.096	−0.053	0.063	−0.039	0.370	−0.029	0.189	−0.081	0.014
BMI	0.006	0.194	0.006	0.173	0.009	0.007	0.009	0.008	−0.008	0.002	−0.008	0.002
pAHI	0.001	0.638	−0.001	0.792	0.000	0.752	0.000	0.934	0.000	0.641	−0.001	0.258
pAHI × gender			0.002	0.380			−0.001	0.651			0.003	0.034

L1–4 BMD, L1–4 bone mineral density; FN BMD, femoral neck BMD; TBS, trabecular bone score L1–4; BMI, body mass index p, peripheral arterial tone (PAT); pAHI, PAT-derived apnea–hypopnea index; pAHI × gender, interaction between pAHI and gender.
Gender, men = 0, women = 1.
[a] n = 80.

Table 4

Multiple regression analysis between factors, pODI and L1-4 BMD, FN BMD and TBS.

	L1–4 BMD[a]				FN BMD				TBS[a]			
	Model 1		Model 2		Model 1		Model 2		Model 1		Model 2	
	b	p value	b	p value	b	p value	b	p value	b	p value	b	p value
Age	−0.003	0.096	−0.003	0.110	−0.003	0.008	−0.004	0.008	−0.003	0.003	−0.003	0.004
Gender	−0.064	0.093	−0.087	0.087	−0.058	0.043	−0.051	0.176	−0.034	0.122	−0.072	0.013
BMI	0.006	0.132	0.006	0.129	0.009	0.004	0.009	0.004	−0.007	0.003	−0.007	0.003
pODI	0.000	0.997	−0.003	0.514	−0.001	0.403	0.000	0.968	0.000	0.779	−0.005	0.049
pODI × gender			0.002	0.488			0.000	0.792			0.004	0.046

L1–4 BMD, L1–4 bone mineral density; FN BMD, femoral neck BMD; TBS, trabecular bone score L1–4; BMI, body mass index; p, peripheral arterial tone (PAT); pODI, PAT-derived oxygen desaturation index; pODI × gender, interaction between pODI and gender.
Gender, men = 0, women = 1.
[a] n = 80.

Table 5

Multiple regression analysis between age, BMI, pAHI/pODI and TBS in subjects stratified by gender and menopausal status.

	Men (n = 33)		Women (n = 47)		Postmenopausal women (n = 33)		Premenopausal women (n = 14)	
Characteristics	b	p value	b	p value	b	p value	b	p value
pAHI as an independent variable								
Age	0	0.803	−0.005	< 0.001	−0.006	0.003	0.000	0.999
BMI	−0.003	0.443	−0.010	0.004	−0.010	0.006	−0.020	0.068
pAHI	−0.002	0.034	0.002	0.059	0.004	0.003	0.000	0.903
pODI as an independent variable								
Age	0.000	0.911	−0.005	0.001	−0.007	0.003	0.000	0.927
BMI	−0.003	0.325	−0.009	0.007	−0.009	0.011	−0.019	0.075
pODI	−0.003	0.021	0.002	0.161	0.004	0.008	−0.001	0.697

BMI, body mass index; p, peripheral arterial tone (PAT); pAHI, PAT-derived apnea–hypopnea index; pODI, PAT-derived oxygen desaturation index; TBS, trabecular bone score L1–4.

in T2DM patients. In this particular group of participants, BMD at L1-4 and FN did not correlate with OSA severity (pAHI and pODI). Interestingly, TBS associated with severity of OSA with the opposite direction between two genders. Independent of age and BMI, higher pAHI and higher pODI significantly predicted lower TBS in men, but higher pAHI borderline significantly predicted higher TBS in women ($p = 0.059$). Of note, post hoc analysis in postmenopausal women demonstrated a significant association between more severe OSA (both pAHI and pODI) and higher TBS. This information highlights the complex relationship between OSA severity, gender and TBS in T2DM.

The reason for the opposite direction of the association between TBS and OSA severity among men and women is not clear as this has not been explored previously, especially in T2DM. In mice, one study explored the effect of intermittent hypoxia on BMD in different genders and gonadal status [26]. In that study, intermittent hypoxia did not modify BMD in normal mice of both genders and in males with orchiectomy-induced osteoporosis [26]. In humans, previous studies have investigated the relationship between OSA severity and BMD, but not TBS. However, the results were not always concordant. Studies which included more men tended to find detrimental effects of OSA on bone while those including more women tended to find the contrary. For example, the recent study in Taiwan (83% men) demonstrated that patients with OSA (1377 patients) were 2.74 times higher risk of osteoporosis than age- and gender-matched controls without OSA, after adjusting for age, gender and diabetes status [15]. Similarly, a study exclusively in men reported that subjects with OSA (n = 21, mean age 54 years) had lower lumbar spine and FN BMD compared to 26 healthy age-and gender-matched controls [17]. In addition, a study in 50 men found that urinary concentrations of cross-linked C-terminal telopeptide of type I collagen, a bone resorption marker, were higher in those with more severe OSA and decreased significantly after 3 months of treatment with continuous positive airway pressure [16]. On the

contrary, the study of Sforza et al. in 2013 which included more women than men participants (n = 832, 59% women) revealed higher femoral and spinal BMD in those with OSA than those without OSA [19]. In 2016, Sforza confirmed a protective effect of OSA on BMD in another study in which 57.5% of the participants were women (total n = 461, mean age 68.7 years) [18]. It was found that participants with osteoporosis had less severe OSA than those without osteoporosis, and that increasing OSA severity was an independent predictor of higher lumbar and femoral BMD [18]. Besides differences in OSA severity, BMI and intrinsic bone property between diabetic and non-diabetic participants, it is possible that these contradictory results were in part contributed by different gender distribution in each study. Our finding of different associations between pAHI and pODI and TBS in men and women may help explain the discordant results of previous studies.

Mechanistically, hypoxia, as a result of OSA, has been reported to relate with both anabolic and catabolic effects on the skeleton. Some data suggested that hypoxia impaired bone mass and quality through many pathways. First, there are disruption in the balance between osteoclasts and osteoblasts and increased bone remodeling in favor of bone resorption over bone formation [27–29]. Second, hypoxia is harmful to bone by inducing acidosis and inflammation. Acidosis has been shown to activate osteoclasts and inhibit mineral deposition by osteoblasts [30], leading to fractures [31]. Third, oxidative stress associated with hypoxia can lead to increased bone resorption and lower bone mass, possibly through alterations of collagen structure, osteoclast stimulation, and inhibition of osteoblast differentiation and function [32]. Lastly, other proposed mechanisms included sleep restriction [14], increased sympathetic tone associated with OSA [14], alteration in melatonin [33], and other hormonal or comorbid conditions [14]. These evidences support the detrimental effects of hypoxia on bone. However, in contrast to these data, some studies in animal and cell-culture models have suggested a protective effect of OSA on bone. For
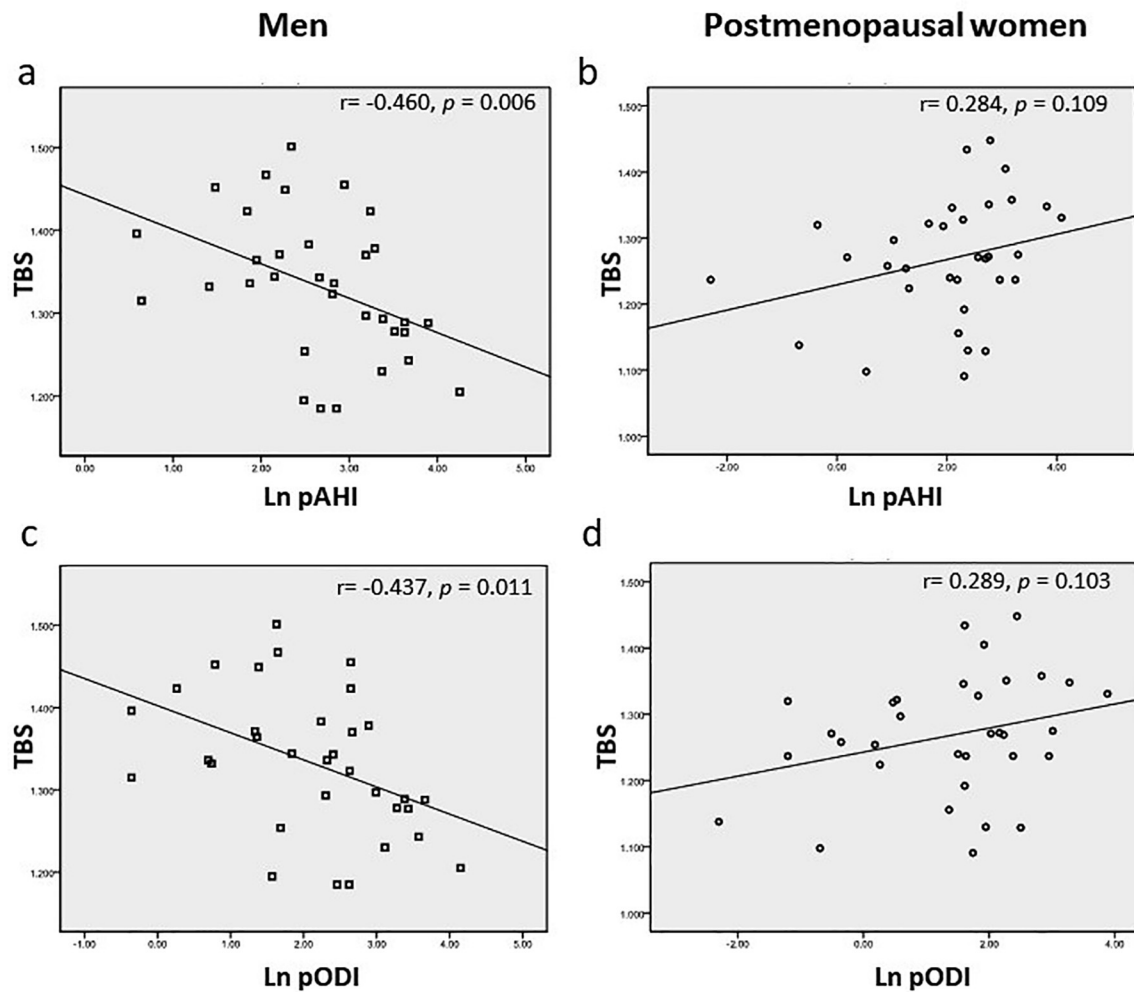
Fig. 1. The association between pAHI or pODI and TBS in men (n = 33) and postmenopausal women (n = 34). pAHI and pODI were not normally distributed and the natural logarithm transformations (Ln) of these variables were used for the analyses. r = Pearson Correlation, p, peripheral arterial tone (PAT); pAHI, PAT-derived apnea–hypopnea index; pODI, PAT-derived oxygen desaturation index; TBS, trabecular bone score L1–4.

example, serum from rats exposed to recurrent hypoxia had higher levels of chemotaxis for mesenchymal stem cells, compared to those of control, which could promote osteoblast differentiation [34]. In addition, osteoblasts and osteocytes cultured under hypoxia had decreased sclerostin expression and increased Wnt signaling, favoring bone formation [35]. Collectively, clinical studies and data from research at cell and animal levels remain conflicting regarding the potential effects of intermittent hypoxia on bone health. The opposite direction of the association between OSA severity (pAHI) and the measurement of intermittent hypoxia (pODI) and TBS among men and postmenopausal women suggested that gender may play a role in the different effects of OSA on bone health, especially in T2DM. In addition, it is well established that hypogonadism is associated with both OSA and T2DM. Although we have excluded people with a diagnosis of hypogonadism but we did not measure testosterone. This could be a confounding factor. Future controlled studies involving a greater number of participants and consideration of gonadal status assessment, especially in men, are warranted. Research should involve an interventional study exploring the effects of OSA treatment on bone metabolism, especially in T2DM patients.

To date, TBS may serve as an indicator of vertebral skeletal deterioration in patients with T2DM independent of BMD. However, additional tools are warranted to assess abnormal bone quality. For example, increased cortical porosity in postmenopausal women with T2DM was demonstrated by performing high resolution peripheral

quantitative computed tomography (HRpQCT) [35,36]. In addition to increased cortical porosity, high levels of advanced glycation end products and an imbalance between bone resorption and bone formation could play roles in skeletal fragility in T2DM [36].

The strength of our study is that, to our knowledge, this is the first study to demonstrate that OSA severity in T2DM participants is associated with deterioration of bone microarchitecture in men but may improve bone microarchitecture in women. However, there are also limitations. The number of participants was relatively small. A post-hoc power of test was estimated for the multiple regression analysis of pAHI and TBS in subjects stratified by gender (Table 5). The power of the test in women (n = 47) and postmenopausal women (n = 33) were 96.1% and 97.1%, respectively, but it was only 76.4% for detecting the effect of pAHI on TBS in men. The participants had relatively well-controlled diabetes, which may not be a true representation of the patient population. In addition, OSA was not diagnosed using a gold standard polysomnography. Assessment of the severity of OSA by WatchPAT may under-estimate and sometimes overestimate. Because of limited availability of quantitative computed tomography or HRpQCT in our country, we did not assess bone quality by these images in parallel with TBS. As the study was cross-sectional, a causal relationship between OSA and deterioration of bone microarchitecture could not be derived. The detail of smoking status, for example smoking intensity in pack-year and duration of time since subjects quit for ex-smokers, was lacking. There was an imbalance in duration of diabetes among genders

and may partly explain the differences in the association between TBS and OSA severity. However, when compared between men and post-menopausal women, there was no difference in duration of diabetes (10 (5.5–19.5), and 9.50 (4–15) years, $p = 0.288$). Finally, fracture outcomes were not assessed.

Conclusions

There is a complex relationship between OSA severity, gender and bone quality in T2DM. A combination of OSA and T2DM might further deteriorate bone quality and increase fracture risk in men but tend to preserve bone microarchitecture in women. Interventional studies exploring the impact of OSA treatment on bone could help elucidate the underlying mechanisms, and support the benefits of OSA screening and treatment, especially in men with T2DM.

Declarations

Ethics approval and consent to participate

All participants gave written informed consent. The protocol was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand.

Competing interests

SR received speaker honoraria from Sanofi Aventis, Novo Nordisk, and Medtronic; and a research grant from Merck. HN received speaker honoraria from Sanofi Aventis, Novo Nordisk, MSD, Takeda and Astra Zeneca. NS, CS, SS, LC, NC and BO have nothing to disclose.

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Authors' contributions

HN and SR conceived of the study, participated in its design and coordination, performed the statistical analysis and were major contributors in writing the manuscript. NS and SS participated in coordination. CS performed BMD and TBS measurement. LC carried out laboratory (metabolic bone) assessment. NC and BO conceived of the study and participated in its design. All authors read and approved the final manuscript."

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