

## RESEARCH

# Thyroid and bone turnover markers in type 2 diabetes: results from the METAL study

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## Abstract

**Objective:** We aimed to evaluate whether thyroid hormones, autoimmune and thyroid homeostasis status were related to bone turnover in type 2 diabetes.

**Methods:** The data were obtained from a cross-sectional study, the METAL study. In this study, 4209 participants (2059 men and 2150 postmenopausal women) with type 2 diabetes were enrolled. Thyroid function, thyroid antibodies and three bone turnover markers (BTMs), including a large N-mid fragment of osteocalcin (N-MID osteocalcin),  $\beta$ -C-terminal cross-linked telopeptides of type I collagen ( $\beta$ -CTX) and procollagen type I N-terminal propeptide (P1NP), were measured. Thyroid homeostasis parameters, including the sum activity of step-up deiodinases (SPINA-G<sub>D</sub>), thyroid secretory capacity (SPINA-G<sub>T</sub>), Jostel's TSH index (TSHI) and the thyrotroph thyroid hormone resistance index (TTSI), were calculated. The associations of thyroid parameters with BTMs were analyzed using linear regression.

**Results:** Free and total triiodothyronine were positively associated with N-MID osteocalcin and P1NP in both sexes and positively associated with  $\beta$ -CTX in postmenopausal women. Thyroid-stimulating hormone was negatively associated with  $\beta$ -CTX in postmenopausal women, and free thyroxine was negatively associated with N-MID osteocalcin and P1NP in men. SPINA-G<sub>D</sub> was positively associated with N-MID osteocalcin and P1NP in both sexes. There was a positive relationship of SPINA-G<sub>T</sub> with  $\beta$ -CTX, a negative relationship of TTSI with  $\beta$ -CTX, and a negative relationship of TSHI with  $\beta$ -CTX and P1NP in postmenopausal women.

**Conclusions:** Among men and postmenopausal women with type 2 diabetes, significant associations were observed between N-MID osteocalcin,  $\beta$ -CTX and P1NP with thyroid function and thyroid homeostasis. Further prospective studies are warranted to understand the causal relationship and underlying mechanism.

## Key Words

- ▶ bone turnover marker
- ▶ thyroid hormone
- ▶ thyroid homeostasis parameters
- ▶ type 2 diabetes mellitus

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## Introduction

Type 2 diabetes mellitus (T2DM) is associated with an increased fracture risk (1, 2, 3), which has aroused great concern. One of the most commonly used indicators to assess fracture risk is the bone mineral density (BMD) estimated by dual energy X-ray absorptiometry (4). However, compared with controls, patients with T2DM have a higher BMD, which cannot explain the observed

increased fracture risk (5). Thus, many studies have investigated bone turnover status in patients with T2DM.

Bone turnover status may be assessed by measuring biochemical markers and bone turnover markers (BTMs), which ideally reflect bone resorption and formation processes (1). Previous studies have indicated that BTMs of both bone formation and bone resorption are decreased

in patients with diabetes (1, 6, 7), which suggested that a state of low bone turnover in diabetes mellitus may lead to increased fracture risk. The International Osteoporosis Foundation and the International Federation of Clinical Chemistry and Laboratory Medicine recommend that a marker of bone formation (serum procollagen type I N propeptide, s-P1NP) and a marker of bone resorption (serum C-terminal telopeptide of type I collagen, s-CTX) be used as reference analytes for BTMs (8). Osteocalcin (OC) is also used as a bone formation marker in clinical and research settings (8).

There has been growing interest in the effect of thyroid dysfunction and even mild or transient disturbances in thyroid status on bone physiology and pathology (9, 10, 11). Moreover, the hypothalamic–pituitary–thyroid axis also plays a key role in skeletal development, acquisition of peak bone mass and regulation of the bone turnover process (12).

To the best of our knowledge, there have been few reports on the relationship of BTMs with thyroid hormones (9), thyroid homeostasis parameters and thyroid antibodies in patients with T2DM. We hypothesized that thyroid hormones, thyroid autoimmune status, hypothalamic–pituitary–thyroid axis is involved in the regulation of bone formation and resorption process in T2DM. In this observational explorative study, we evaluated whether thyroid hormones, including thyroid-stimulating hormone (TSH), free and total thyroxine (FT<sub>4</sub>, TT<sub>4</sub>), free and total triiodothyronine (FT<sub>3</sub>, TT<sub>3</sub>), thyroid antibodies, including thyroid peroxidase antibody (TPOAb) and thyroglobulin antibody (TgAb), and four calculated thyroid homeostasis parameters, including the sum activity of step-up deiodinases (SPINA-G<sub>D</sub>), thyroid secretory capacity (SPINA-G<sub>T</sub>), Jostel's TSH index (TSHI) and the thyrotroph thyroid hormone resistance index (TTSI), were related to three BTMs, including large N-mid fragment of OC (N-MID OC),  $\beta$ -CTX and P1NP, in a population with T2DM.

## Methods

### Study design and participants

The data of this study were obtained from a cross-sectional study, the METAL study (Environmental Pollutant Exposure and Metabolic Diseases in Shanghai, [www.chictr.org.cn](http://www.chictr.org.cn), ChiCTR1800017573) (13, 14), which was designed to investigate the association between exposure to heavy metals and diabetes complications in Chinese adults with diabetes in 2018. Participants were from

the seven communities in Shanghai, China, and were randomly selected from half of the patients with T2DM in the registration platform of each community health care center ( $n = 4937$ ). We excluded participants who were missing laboratory results ( $n = 8$ ) or questionnaire data ( $n = 116$ ), missing BTMs ( $n = 45$ ), missing thyroid parameters ( $n = 4$ ), had a history of thyroid surgery or known thyroid dysfunction, that is, all potential users of drugs for thyroid dysfunction ( $n = 274$ ), had a history of glucocorticoid or amiodarone treatment ( $n = 177$ ), had an estimated glomerular filtration rate (eGFR)  $<30$  mL/min/1.73 m<sup>2</sup> ( $n = 18$ ) or were premenopausal women ( $n = 86$ ). Finally, 4209 participants (2059 men and 2150 postmenopausal women) were involved in this study.

All participants provided written informed consent before data collection. The study protocol was approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 1975 Declaration of Helsinki, as revised in 2008.

### Laboratory measurements

Fasting blood samples for laboratory assays were obtained by venipuncture from 07:00 to 10:00 h. Blood samples were stored at 2–8°C when collected and shipped to local central laboratories, which were certified by the College of American Pathologists, within 2–4 h of collection. On the day of blood sample collection, as soon as the blood samples arrived in the laboratory, serum separation and sample testing began.

Serum TSH, FT<sub>3</sub>, FT<sub>4</sub>, TT<sub>3</sub>, TT<sub>4</sub>, TPOAb and TgAb were measured by electrochemiluminescence (Roche, E601). The normal reference ranges for TSH, FT<sub>3</sub>, FT<sub>4</sub>, TT<sub>3</sub>, TT<sub>4</sub>, TPOAb and TgAb were 0.27–4.20 mIU/L, 3.10–6.80 pmol/L, 12.00–22.00 pmol/L, 1.30–3.10 nmol/L, 66.00–181.00 nmol/L, 0–34.00 U/mL and 0–115.00 U/mL, respectively. The interassay coefficients of variation were as follows: 8.33% (TSH), 8.33% (FT<sub>3</sub>), 8.33% (FT<sub>4</sub>), 8.33% (TT<sub>3</sub>), 6.67% (TT<sub>4</sub>), 10.00% (TPOAb) and 10.00% (TgAb). The intra-assay coefficients of variation were as follows: 6.25% (TSH), 6.25% (FT<sub>3</sub>), 6.25% (FT<sub>4</sub>), 6.25% (TT<sub>3</sub>), 5.00% (TT<sub>4</sub>), 7.50% (TPOAb) and 7.50% (TgAb).

BTMs, including N-MID OC,  $\beta$ -CTX and P1NP, were detected with a chemiluminescence method (Roche E602). The inter-assay coefficients of variation were as follows: 3.30% (P1NP), 1.81% (N-MID OC) and 7.60% ( $\beta$ -CTX). The intra-assay coefficients of variation

were as follows: 3.0% (P1NP), 0.80% (N-MID OC) and 5.50% ( $\beta$ -CTX).

HbA1c was measured via HPLC (MQ-2000PT, Medconn, Shanghai, China). Creatinine (Cr) was measured by a Beckman Coulter AU 680 (Brea, USA). Serum 25(OH)D was measured using the chemiluminescence method (SIEMENS ADVIA Centaur XP, Siemens).

### Clinical parameters

A questionnaire about sociodemographic characteristics, medical history, family history, and lifestyle factors was adopted during the interview. The same group of trained and experienced personnel in the SPECT-China study (Survey on Prevalence in East China for Metabolic Diseases and Risk Factors, ChiCTR-ECS-14005052, [www.chictr.org.cn](http://www.chictr.org.cn)) (15, 16) conducted the interviews and clinical examinations, including measurements of weight, height and blood pressure, according to a standard protocol.

Current smoking was defined as having smoked at least 100 cigarettes in one's lifetime and currently smoking cigarettes (17). Anti-thyroid antibody positivity (ATA+) was defined as serum TPOAb positivity (TPOAb+) (>34.00 U/mL) and/or TgAb positivity (TgAb+) (>115.00 U/mL).

Two structural parameters of thyroid homeostasis, SPINA-G<sub>D</sub> and SPINA-G<sub>T</sub>, and two pituitary thyrotropic function indices, TSHI and TTSI, were calculated using the following equations according to a previous study (18):

$$SPINA-G_D = \left( 8 \times 10^{-6} \times (500 + [FT_4]) \times [TT_3] \right) / \left( 0.026 \times [FT_4] \right)$$

$$SPINA-G_T = \left( 1.1 \times 10^{-6} \times (2.75 + [TSH]) \times [TT_4] \right) / \left( 0.1 \times [TSH] \right)$$

$$TSHI = \ln([TSH]) + 0.1345 \times [FT_4]$$

$$TTSI = \left( 100 \times [TSH] \times [FT_4] \right) / 22$$

BMI was calculated as weight in kilograms divided by height in meters squared. The eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation for 'Asian origin' (19).

### Statistical analysis

Survey analyses were performed with IBM SPSS Statistics. All analyses were two-sided. A *P*-value <0.05 was taken to indicate a significant difference. Normally and non-normally distributed continuous variables were expressed as the mean  $\pm$  s.d. and medians (interquartile range), respectively. Categorical variables are presented as

percentages. Normally distributed continuous variables were compared using Student's *t*-test. The Mann-Whitney *U* test was used for non-normally distributed continuous variables. The Pearson's chi-square test was used for dichotomous variables. Associations of thyroid function, ATA+ and thyroid homeostasis parameters with three BTMs, including N-MID OC,  $\beta$ -CTX and P1NP, were analyzed using linear regression models with each measure as the outcome. N-MID OC,  $\beta$ -CTX and P1NP were ln-transformed for normal distribution before linear regression analysis. The regression models were adjusted for age, BMI, duration of diabetes, current smoking, concentration of HbA1c, 25(OH)D and Cr, and the use of metformin and thiazolidinediones. The results were expressed as B values and 95% CIs. Considering the significant sex differences in bone metabolism between men and women, all statistical analyses were performed separately for men and postmenopausal women.

## Results

### Clinical characteristics of the participants

This study recruited 4209 participants with T2DM, including 2059 men and 2150 postmenopausal women. The mean ages of men and postmenopausal women were 67.43 and 67.65 years old, respectively. Their median duration of diabetes was 8 years. There was no significant difference between men and postmenopausal women in the use of metformin or thiazolidinedione. Compared to postmenopausal women, men had higher levels of HbA1c, Cr, 25(OH)D, FT<sub>3</sub>, FT<sub>4</sub> and SPINA-G<sub>T</sub>, lower levels of TSH, TT<sub>4</sub>, TTSI, TSHI and BTMs (N-MID OC,  $\beta$ -CTX and P1NP) and a lower prevalence of TPOAb+, TgAb+ and ATA+ (Table 1). In addition, compared to participants with ATA-, ATA+ participants had higher levels of TSH, SPINA-G<sub>D</sub>, TTSI, TSHI, N-MID OC,  $\beta$ -CTX and P1NP and lower levels of HbA1c, Cr, FT<sub>3</sub>, FT<sub>4</sub> and SPINA-G<sub>T</sub> (Table 2).

### Association of BTMs with thyroid function and ATA+ in type 2 diabetes

We analyzed the associations of thyroid function and ATA+ with three BTMs using linear regression models. The regression models were adjusted for related factors, including age, BMI, duration of diabetes, current smoking, concentrations of HbA1c, 25(OH)D and Cr, and the use of metformin and thiazolidinediones.

**Table 1** Characteristics of the study participants.

Characteristic	Men	Postmenopausal women	P
<i>n</i>	2059	2150	-
Age (years)	67.43 ± 8.76	67.65 ± 7.71	0.399
Duration of diabetes (years)	8.00 (12.00)	8.00 (12.00)	0.605
HbA1c (%)	7.30 (1.60)	7.10 (1.50)	<0.001
BMI (kg/m <sup>2</sup> )	25.01 ± 3.30	24.89 ± 3.79	0.255
Cr (μmol/L)	76.00 (20.00)	57.00 (15.00)	<0.001
25(OH)D (nmol/L)	42.69 ± 14.68	38.93 ± 13.35	<0.001
Current smoking (%)	36.5	2.3	<0.001
Metformin in use (%)	38.2	38.7	0.728
Thiazolidinediones in use (%)	3.1	3.2	0.781
Thyroid-related parameters			
TSH (mIU/L)	2.32 (1.59)	2.76 (1.96)	<0.001
FT <sub>3</sub> (pmol/L)	4.78 ± 0.59	4.50 ± 0.56	<0.001
FT <sub>4</sub> (pmol/L)	16.95 ± 2.28	16.58 ± 2.25	<0.001
TT <sub>3</sub> (nmol/L)	1.66 ± 0.30	1.64 ± 0.28	0.054
TT <sub>4</sub> (nmol/L)	98.94 ± 17.88	103.06 ± 17.47	<0.001
TPOAb+ (%)	5.1	10.0	<0.001
TgAb+ (%)	3.2	8.9	<0.001
ATA+ (%)	6.2	14.2	<0.001
Thyroid homeostasis parameters			
SPINA-G <sub>D</sub> (nmol/s)	15.26 ± 3.23	15.42 ± 3.01	0.085
SPINA-G <sub>T</sub> (pmol/s)	2.38 (1.05)	2.25 (1.05)	<0.001
TTSI (mIU/L)	179.75 (123.04)	207.83 (145.02)	<0.001
TSHI	3.13 ± 0.58	3.22 ± 0.59	<0.001
Bone turnover markers			
N-MID osteocalcin (ng/mL)	9.40 (4.30)	12.30 (6.03)	<0.001
β-CTX (ng/mL)	0.17 (0.11)	0.22 (0.12)	<0.001
P1NP (ng/mL)	34.53 (16.48)	44.53 (23.01)	<0.001

Data were summarized as median (interquartile range) or mean ± s.d. for continuous variables or a proportion for categorical variables. Normally distributed continuous variables were compared using Student's *t*-test. The Mann-Whitney *U* test and the Kruskal-Wallis test were used for non-normally distributed continuous variables. Pearson's chi-square test was used for dichotomous variables. ATA+ was defined as the serum TPOAb+ (>34.00 U/mL) and/or TgAb+ (>115.00 U/mL).

ATA, anti-thyroid antibody; Cr, creatinine; eGFR, estimated glomerular filtration rate; FT<sub>3</sub>, free triiodothyronine; FT<sub>4</sub>, free thyroxine; N-MID osteocalcin, large N-mid fragment of osteocalcin; P1NP, procollagen type I N-terminal propeptide; SPINA-G<sub>T</sub>, thyroid's secretory capacity; SPINA-G<sub>D</sub>, sum activity of peripheral deiodinases; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone; TSHI, Jostel's TSH index; TT<sub>3</sub>, total triiodothyronine; TT<sub>4</sub>, total thyroxine; TTSI, thyrotroph thyroid hormone resistance index; β-CTX, β-C-terminal cross-linked telopeptides of type I collagen.

We found that TSH levels were negatively associated with β-CTX only in postmenopausal women (B = -0.047, 95% CI = -0.077 to -0.016). FT<sub>4</sub> levels were negatively associated with N-MID OC (B = -0.007, 95% CI = -0.014 to -0.001) and P1NP (B = -0.013, 95% CI = -0.021 to -0.006) in men. FT<sub>3</sub> and TT<sub>3</sub> were positively associated with β-CTX only in postmenopausal women (FT<sub>3</sub>: B = 0.085, 95% CI = 0.050-0.120; TT<sub>3</sub>: B = 0.092, 95% CI = 0.023-0.161) and positively associated with N-MID OC and P1NP in both sexes (N-MID OC: men: FT<sub>3</sub>: B = 0.064, 95% CI = 0.036-0.092; TT<sub>3</sub>: B = 0.143, 95% CI = 0.091-0.194; postmenopausal women: FT<sub>3</sub>: B = 0.097, 95% CI = 0.069-0.124; TT<sub>3</sub>: B = 0.173, 95% CI = 0.119-0.227 and P1NP: men: FT<sub>3</sub>: B = 0.049, 95% CI = 0.018-0.081; T<sub>3</sub>: B = 0.174, 95% CI = 0.112-0.235; postmenopausal women: FT<sub>3</sub>: B = 0.096, 95% CI = 0.065-0.128; TT<sub>3</sub>: B = 0.174, 95% CI = 0.112-0.235) (Fig. 1).

### Association of BTMs with thyroid homeostasis parameters in type 2 diabetes

We then analyzed the associations of thyroid homeostasis parameters with three BTMs using linear regression models (Fig. 1). After full adjustment, we found that SPINA-G<sub>D</sub> was positively associated with N-MID OC (B = 0.014, 95% CI = 0.009-0.018) and P1NP (B = 0.015, 95% CI = 0.010-0.021) in men. For postmenopausal women, more significant differences were found. For example, SPINA-G<sub>D</sub> levels were positively associated with N-MID OC (B = 0.012, 95% CI = 0.007-0.017) and P1NP (B = 0.017, 95% CI = 0.011-0.023), β-CTX was positively associated with SPINA-G<sub>T</sub> (B = 0.055, 95% CI = 0.010-0.100) and negatively associated with TTSI (B = -0.049, 95% CI = -0.081 to -0.017) and TSHI levels were negatively associated with β-CTX (B = -0.049,

**Table 2** Characteristics of the study participants according to the presence of ATA positivity.

Characteristic	ATA–	ATA+	P
n	3777	432	-
Men/women (n)	1932/1845	127/305	-
Age (years)	67.55 ± 8.26	67.50 ± 8.10	0.923
HbA1c (%)	7.20 (1.60)	7.00 (1.50)	0.023
BMI (kg/m <sup>2</sup> )	24.94 ± 3.55	24.99 ± 3.60	0.820
Cr (μmol/L)	67.00 (23.00)	63.00 (20.75)	<0.001
25(OH)D (nmol/L)	40.78 ± 14.19	40.72 ± 13.77	0.939
TSH (mIU/L)	2.48 (1.75)	3.04 (2.45)	<0.001
FT <sub>3</sub> (pmol/L)	4.65 ± 0.59	4.54 ± 0.59	<0.001
FT <sub>4</sub> (pmol/L)	16.80 ± 2.22	16.42 ± 2.64	0.004
TT <sub>3</sub> (nmol/L)	1.65 ± 0.29	1.65 ± 0.32	0.710
TT <sub>4</sub> (nmol/L)	100.98 ± 17.63	101.59 ± 19.19	0.503
SPINA-G <sub>D</sub> (nmol/s)	15.29 ± 3.02	15.84 ± 3.86	0.004
SPINA-G <sub>T</sub> (pmol/s)	2.33 (1.06)	2.14 (1.06)	<0.001
TTSI	189.29 (128.84)	223.19 (172.59)	<0.001
TSHI	3.16 ± 0.58	3.30 ± 0.64	<0.001
N-MID osteocalcin (ng/mL)	10.70 (5.50)	11.70 (5.90)	<0.001
β-CTX (ng/mL)	0.19 (0.12)	0.20 (0.14)	0.012
P1NP (ng/mL)	38.97 (21.41)	42.55 (21.52)	<0.001

Data were summarized as median (interquartile range) or mean ± s.d. for continuous variables or a proportion for categorical variables. Normally distributed continuous variables were compared using Student's *t*-test. The Mann–Whitney *U* test and the Kruskal–Wallis test were used for non-normally distributed continuous variables.

ATA, anti-thyroid antibody; Cr, creatinine; eGFR, estimated glomerular filtration rate; FT<sub>3</sub>, free triiodothyronine; FT<sub>4</sub>, free thyroxine; N-MID osteocalcin, large N-mid fragment of osteocalcin; P1NP, procollagen type I N-terminal propeptide; SPINA-G<sub>T</sub>, thyroid's secretory capacity; SPINA-G<sub>D</sub>, sum activity of peripheral deiodinases; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone; TSHI, Jostel's TSH index; TT<sub>3</sub>, total triiodothyronine; TT<sub>4</sub>, total thyroxine; TTSI, thyrotroph thyroid hormone resistance index; β-CTX, β-C-terminal cross-linked telopeptides of type I collagen.

95% CI = -0.077 to -0.013) and P1NP (B = -0.032, 95% CI = -0.061 to -0.003).

### Association of BTMs with thyroid function and thyroid homeostasis parameters by the presence of ATA positivity

In this study, among all the participants, 6.2% of men and 14.2% of women had ATA positivity. After full adjustment, we evaluated the associations between BTMs and thyroid parameters among the participants with or without ATA+. For men, we were surprised to find that significant associations between BTMs and thyroid parameters were only found in men without ATA+. For women, regardless of whether or not the participants were ATA+, FT<sub>3</sub> and TT<sub>3</sub> levels were positively associated with three BTMs. However, for other thyroid parameters, significant associations

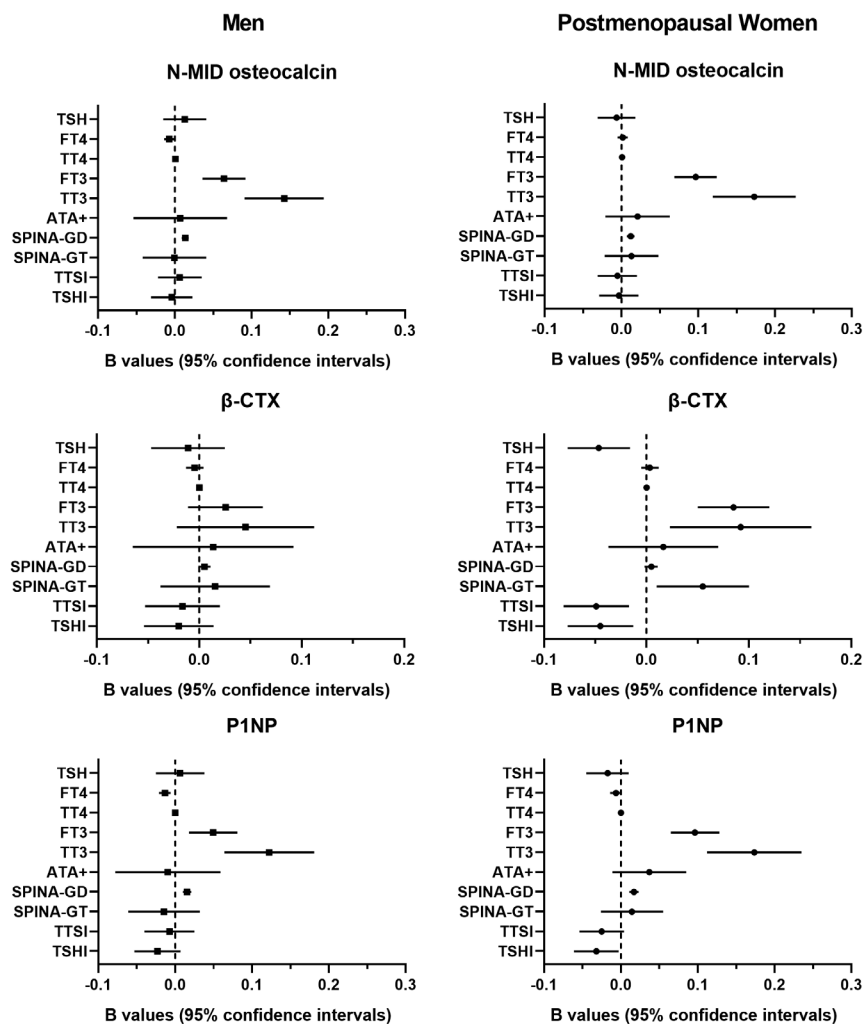
between BTMs and thyroid parameters were also found only among women without ATA+ (Table 3).

### Discussion

Several studies have found that BTMs can predict fracture risk independently of BMD in men and postmenopausal women (8). Recently, some evidence has suggested that BTMs, such as CTX, OC and P1NP, of patients with T2DM are lower than those of healthy controls (6, 20, 21, 22, 23). Thus, the main purpose of this study was to understand the factors that may be involved in the changes in BTMs in patients with T2DM, especially the regulation by thyroid-related parameters. This study mainly discusses three BTMs, that is, P1NP, N-MID OC and β-CTX, which have been recommended for use in epidemiological studies as reference markers of bone formation and resorption (8). Several reports have suggested that anti-thyroid dysfunction treatment may influence the bone metabolism. Long-term levothyroxine substitution therapy, including treatment for hypothyroidism, and TSH suppressive levothyroxine doses in the therapy of thyroidectomy may influence BTMs (24, 25). On the other hand, bone loss could be reversed by the treatment of hyperthyroidism (26). Thus, considering the effects of anti-thyroid dysfunction treatment on BTMs, participants who had a history of thyroid surgery or known thyroid dysfunction, that is, all potential users of drugs for thyroid dysfunction were excluded in this study. None of the participants in this study had a history of taking anti-thyroid treatment.

In T2DM patients, BTMs may be affected by several factors (6), that is, increasing plasma glucose (6, 27), obesity (or BMI) (28) and renal function (29). Furthermore, antidiabetic drugs (6), such as metformin and thiazolidinediones (30, 31), have been suggested to influence bone turnover with inconsistent results (32, 33, 34). Thus, in this study, all of these above factors were adjusted in regression models. In addition, some of the BTMs, such as CTX, were reported to be decreased by food intake (35, 36). Considering the immediate impact of diet on bone metabolism, blood samples collected in this study were all fasting blood samples.

Thyroid hormones regulate skeletal development, longitudinal growth and bone homeostasis (12). The relationship between thyroid function and BTMs has been evaluated in several cross-sectional and prospective studies with controversial results. Although some reports have suggested that overt hyperthyroidism increases



**Figure 1**

Associations between BTMs and thyroid parameters in patients with type 2 diabetes mellitus by linear regression analysis. The data were expressed as B value and 95% CI. TSH, SPINA-G<sub>r</sub> and TTSI were ln-transformed for normal distribution before linear regression analysis. The regression model was adjusted for age, BMI, duration of diabetes, current smoking, concentration of HbA1c, 25(OH) D and Cr, and the use of metformin and thiazolidinediones.

the rate of both bone formation and resorption (9, 11, 37), hypothyroidism results in a low bone turnover state (38), and subclinical hyper- and hypothyroidism are correlated with bone loss (39, 40, 41). Other studies have failed to confirm these associations (42, 43, 44, 45). TSH was negatively correlated with bone formation markers (N-MID OC) (46), and *in vitro* data showed that TSH inhibits osteoclastogenesis in a murine osteoclast cell line (47). However, in the current study, we found that TSH levels were negatively associated with bone resorption ( $\beta$ -CTX) in postmenopausal women. Our observation results may be explained by the presence of thyrotropin (TSH) receptors (TSHRs) in both osteoclasts and osteoblasts (48). In line with a previous study suggesting that FT<sub>3</sub> was significantly positively correlated with N-MID OC and CTX (46), we found that FT<sub>3</sub> and TT<sub>3</sub> were positively associated with N-MID OC and P1NP in both sexes and positively associated with  $\beta$ -CTX only in postmenopausal

women. T<sub>3</sub> is essential for normal bone growth and bone metabolism. T<sub>3</sub> stimulates bone formation directly through T<sub>3</sub> receptors in osteoblasts and stimulates bone resorption by osteoclasts, perhaps secondarily through osteoblasts (49). Thus, in patients with T2DM, low T<sub>3</sub> levels may be associated with impaired bone formation and bone resorption processes. Moreover, the differential regulation of BTMs in different sexes in the current study may be due to the effect of sex steroids on modulating the interplay between thyroid and bone.

The hypothalamic-pituitary-thyroid axis plays a key role in the regulation of bone turnover (12). To date, no epidemiologic studies have focused on the association between thyroid homeostasis parameters and BTMs among participants with T2DM. By extending the classic concept of separate measurements of thyroid hormone parameters, these thyroid homeostasis parameters add new qualitative and quantitative dimensions to the evaluation of thyroid

**Table 3** Associations between bone turnover markers and thyroid function and thyroid homeostasis parameters by the presence of ATA.

	N-MID osteocalcin			β-CTX			P1NP		
	ATA (-) B (95% CI)	P	ATA (+) B (95% CI)	ATA (-) B (95% CI)	P	ATA (+) B (95% CI)	ATA (-) B (95% CI)	P	ATA (+) B (95% CI)
<b>Men</b>									
TSH	0.015 (-0.015, 0.045)	0.339	-0.014 (-0.096, 0.069)	-0.071 (-0.046, 0.031)	0.713	-0.061 (-0.168, 0.047)	0.003 (-0.030, 0.037)	0.844	0.010 (-0.087, 0.108)
FT <sub>4</sub>	<b>-0.008 (-0.015, -0.001)</b>	<b>0.024</b>	0.000 (-0.021, 0.022)	-0.007 (-0.016, 0.002)	0.119	0.020 (-0.008, 0.048)	<b>-0.014 (-0.022, -0.006)</b>	<b>&lt;0.001</b>	0.002 (-0.024, 0.027)
TT <sub>4</sub>	0.001 (0.000, 0.002)	0.137	-0.001 (-0.004, 0.002)	0.000 (-0.001, 0.001)	0.930	0.000 (-0.004, 0.004)	0.000 (-0.001, 0.001)	0.590	-0.001 (-0.005, 0.003)
FT <sub>3</sub>	<b>0.066 (0.037, 0.095)</b>	<b>&lt;0.001</b>	0.037 (-0.066, 0.140)	0.477	0.294	0.110 (-0.023, 0.243)	<b>0.049 (0.016, 0.082)</b>	<b>0.003</b>	0.057 (-0.064, 0.179)
TT <sub>3</sub>	<b>0.159 (0.104, 0.214)</b>	<b>&lt;0.001</b>	-0.001 (-0.161, 0.159)	0.992	0.077	-0.159 (-0.366, 0.048)	<b>0.134 (0.072, 0.195)</b>	<b>&lt;0.001</b>	0.022 (-0.168, 0.212)
SPINA-G <sub>b</sub>	<b>0.017 (0.012, 0.022)</b>	<b>&lt;0.001</b>	-0.004 (-0.015, 0.008)	<b>0.009 (0.002, 0.016)</b>	<b>0.008</b>	-0.018 (-0.033, 0.003)	<b>0.018 (0.012, 0.024)</b>	<b>&lt;0.001</b>	-0.003 (-0.017, 0.011)
SPINA-G <sub>t</sub>	0.004 (-0.040, 0.048)	0.868	-0.033 (-0.176, 0.109)	0.644	0.694	0.073 (-0.113, 0.259)	-0.009 (-0.058, 0.040)	0.725	-0.054 (-0.223, 0.114)
TTSI	0.007 (-0.024, 0.037)	0.673	-0.012 (-0.104, 0.079)	0.791	0.446	-0.048 (-0.168, 0.071)	-0.012 (-0.046, 0.022)	0.496	0.019 (-0.090, 0.128)
TSHI	-0.005 (-0.033, 0.023)	0.748	-0.017 (-0.109, 0.075)	-0.021 (-0.057, 0.014)	0.239	-0.026 (-0.146, 0.095)	-0.028 (-0.059, 0.004)	0.083	0.017 (-0.093, 0.127)
<b>Women</b>									
TSH	-0.012 (-0.039, 0.016)	0.411	0.003 (-0.049, 0.056)	<b>-0.057 (-0.092, -0.022)</b>	<b>0.001</b>	-0.022 (-0.091, 0.046)	-0.025 (-0.057, 0.007)	0.123	0.000 (-0.060, 0.060)
FT <sub>4</sub>	<b>0.002 (-0.006, 0.009)</b>	<b>0.683</b>	0.004 (-0.011, 0.020)	0.598	0.792	0.010 (-0.010, 0.030)	-0.008 (-0.016, 0.001)	0.066	-0.001 (-0.019, 0.017)
TT <sub>4</sub>	0.001 (0.000, 0.002)	0.167	0.000 (-0.002, 0.002)	0.706	0.862	0.001 (-0.001, 0.004)	0.000 (-0.001, 0.001)	0.545	0.000 (-0.003, 0.002)
FT <sub>3</sub>	<b>0.094 (0.064, 0.125)</b>	<b>&lt;0.001</b>	<b>0.119 (0.049, 0.189)</b>	<b>0.001</b>	<b>&lt;0.001</b>	<b>0.124 (0.032, 0.216)</b>	<b>0.096 (0.061, 0.131)</b>	<b>&lt;0.001</b>	<b>0.109 (0.028, 0.189)</b>
TT <sub>3</sub>	<b>0.171 (0.112, 0.230)</b>	<b>&lt;0.001</b>	<b>0.191 (0.053, 0.329)</b>	<b>0.007</b>	<b>0.041</b>	0.159 (-0.023, 0.340)	<b>0.176 (0.109, 0.243)</b>	<b>&lt;0.001</b>	<b>0.166 (0.007, 0.325)</b>
SPINA-G <sub>b</sub>	<b>0.012 (0.007, 0.018)</b>	<b>&lt;0.001</b>	0.012 (-0.001, 0.024)	0.065	0.165	0.005 (-0.011, 0.021)	<b>0.018 (0.012, 0.024)</b>	<b>&lt;0.001</b>	0.014 (0.000, 0.028)
SPINA-G <sub>t</sub>	0.019 (-0.023, 0.061)	0.373	0.005 (-0.063, 0.073)	0.881	<b>0.024</b>	0.040 (-0.049, 0.129)	0.015 (-0.033, 0.063)	0.536	0.013 (-0.065, 0.091)
TTSI	-0.011 (-0.040, 0.018)	0.456	0.007 (-0.050, 0.064)	0.881	<b>0.001</b>	-0.019 (-0.094, 0.056)	<b>-0.034 (-0.067, -0.001)</b>	<b>0.042</b>	-0.002 (-0.068, 0.063)
TSHI	-0.009 (-0.037, 0.019)	0.533	0.014 (-0.049, 0.077)	0.658	<b>0.002</b>	-0.011 (-0.093, 0.072)	<b>-0.041 (-0.073, -0.009)</b>	<b>0.012</b>	-0.002 (-0.074, 0.070)

ATA+ was defined as TPOAb+ (>34.00 U/mL) and/or TgAb+ (>115.00 U/mL). The regression models were adjusted for age, BMI, duration of diabetes, current smoking, concentration of HbA1c, 25(OH)D and Cr, and the use of metformin and thiazolidinediones. The results were expressed as B values and 95% CIs. N-MID osteocalcin, β-CTX, P1NP, TSH, GT and TTSI were ln-transformed for normal distribution before linear regression analysis.

ATA, anti-thyroid antibody; Cr, creatinine; eGFR, estimated glomerular filtration rate; FT<sub>4</sub>, free thyroxine; N-MID osteocalcin, large N-mid fragment of osteocalcin; P1NP, procollagen type I N-terminal propeptide; SPINA-G<sub>t</sub>, thyroid's secretory capacity; SPINA-G<sub>b</sub>, sum activity of peripheral deiodinases; TgAb, thyroglobulin antibody; TPOAb, thyroid peroxidase antibody; TSH, thyroid-stimulating hormone; TSHI, Jostel's TSH index; TT<sub>3</sub>, total triiodothyronine; TT<sub>4</sub>, total thyroxine; TTSI, thyrotroph thyroid hormone resistance index; β-CTX, β-C-terminal cross-linked telopeptides of type I collagen.

homeostasis status, offer a more integrated and systemic view and deliver important insights into the physiology of pituitary–thyroid feedback control (18). SPINA-G<sub>D</sub>, which reflects the maximum stimulated activity of step-up deiodination (i.e. calculated peripheral deiodinase (DIO) activity), is well-correlated with peripheral DIO activity. Positive associations between calculated peripheral DIO activity and bone formation markers in both sexes may also explain the positive association between TT<sub>3</sub> and bone formation markers in the present population. DIO catalyzes the conversion from inactive T<sub>4</sub> to active T<sub>3</sub>, and most circulating T<sub>3</sub> in blood is derived from peripheral T<sub>4</sub> deiodination (50). SPINA-G<sub>T</sub>, referred to as thyroid capacity, provides an estimate for the maximum secretion rate of the thyroid gland. *In vivo* validation confirmed that SPINA-G<sub>T</sub> is able to clearly differentiate between euthyroidism and functional thyroid disorders of primary origin (51). Unlike TSH, it is unaffected by hypothalamic–pituitary dysfunction. We found a positive relationship between SPINA-G<sub>T</sub> and  $\beta$ -CTX in postmenopausal women. The present observation suggested that changes in thyroid capacity may play a role in bone resorption. TSHI and TTSI were introduced as quantitative markers for pituitary thyrotropic function. Negative relationships of TTSI with  $\beta$ -CTX and TSHI with  $\beta$ -CTX and P1NP were found in postmenopausal women. We hypothesized that pituitary thyrotropic function may also be a possible mechanism of bone metabolism (18).

Different patterns of associations between BTMs and thyroid measures by the status of thyroid autoantibodies suggest that thyroid autoantibodies should be considered in the regulation of bone metabolism by thyroid hormones. Although one study reported that the presence of TPOAb is a potential marker of higher fracture risk in these patients (52), associations between BTMs and TPOAb or TgAb status have rarely been assessed in the human population. In the current observational study, while no direct associations were observed between BTMs and thyroid autoantibody status, the associations with TSH, FT<sub>4</sub>, SPINA-G<sub>D</sub>, SPINA-G<sub>T</sub>, TTSI and TSHI appeared to be influenced by ATA status. Indeed, many significant associations that were observed among the ATA– subjects disappeared among the ATA+ group. The relationship between thyroid antibody and bone metabolism, and its regulatory mechanism are still unclear. More large-scale prospective studies are needed to evaluate these findings.

Although our study had some strengths, including a relatively larger sample size of community-dwelling participants and strong quality control, there were also some limitations. First, this was a cross-sectional study;

thus, a causal relationship could not be identified between BTMs and thyroid function and thyroid homeostasis parameters. Larger cohort studies are needed. Secondly, although determination of bone turnover in diabetes may require bone biopsies to reveal microscopic changes, bone tissue biopsies are difficult to obtain in such a large population. Thirdly, combinations of multiple antidiabetic drug treatments are very common in patients with T2DM. In this study, although the effects of metformin and thiazolidinedione on bone metabolism were considered, the effects of multiple antidiabetic drugs on bone metabolism were not discussed in this study. Fourthly, calcium concentration, parathyroid hormone concentration and BMD were not measured in this study and should be measured in future research. Considering the limitation of the small sample size of fracture history ( $n = 27$ ), fracture was not included in the statistical analysis in this study. Moreover, although the participants with a history of thyroid surgery or known thyroid dysfunction were recorded, what kind of anti-thyroid dysfunction treatment and the dosing the patient was currently taking were not recorded. The issue on the association of high and low dosing of levothyroxine substitution therapy with bone metabolism would be explored in future study.

## Conclusion

Among men and postmenopausal women with T2DM, significant associations of N-MID OC,  $\beta$ -CTX and P1NP with thyroid function and thyroid homeostasis were observed. Further prospective and animal studies are warranted to understand the causal relationship and underlying mechanism.

### 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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#### Author contribution statement

Yingli Lu and Yi Chen designed, performed and supervised this investigation, and had full access to all of the data and took responsibility for the integrity of the data and the accuracy of the data analysis. Yi Chen performed this investigation, analyzed the data, contributed to the discussion, interpretation of the data and the manuscript writing. Wen Zhang, Chi Chen, Yuying Wang and Ningjian Wang provided technical or material support, and contributed to the discussion. All authors read and approved the final manuscript.

#### Data availability

The data used to support the findings of this study are included within the article.

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