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ORIGINAL ARTICLE

Serum fibroblast growth factor 23 and mineral metabolism in patients with euthyroid Graves' diseases: a case-control study

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Abstract

Summary This study investigated the alterations of mineral metabolism in patients with Graves' disease (GD) who achieved euthyroidism. They had higher fibroblast growth factor 23 (FGF23) and phosphorus as compared with healthy subjects. Serum FGF23 was negatively correlated with serum phosphorus. These indicated abnormal mineral metabolism even after 1.6 years of euthyroid status.

Introduction FGF23 is involved in the mineral homeostasis, especially the regulation of serum phosphorus. Graves' disease (GD) is associated with accelerated bone turnover, hyperphosphatemia, and elevated serum FGF23. Evidence suggested that serum FGF23 decreased after a 3-month treatment of GD. However, it remains unclear whether serum FGF23, serum phosphorus, and other markers of mineral metabolism will be normalized after euthyroid status achieved.

Methods A total of 62 patients with euthyroid GD and 62 healthy control subjects were enrolled, and the median duration of euthyroid status was 1.6 years. Endocrine profiles including thyroid function test, autoantibodies, serum FGF23, and bone turnover markers were obtained and compared between the two groups.

Results Euthyroid GD patients had significantly higher serum FGF23 and phosphorus, and lower 25-hydroxyvitamin D (25(OH)D) and intact parathyroid hormone (iPTH) levels as compared with the control group. Serum FGF23 was significantly and negatively correlated with phosphorus level after adjusted for age, gender, calcium, iPTH, and 25(OH)D in the euthyroid GD group.

Conclusion Serum phosphorus and FGF23 levels remain higher in GD patients even after euthyroid status has been achieved for a median of 1.6 years. Serum FGF23 was negatively correlated with serum phosphorus in euthyroid GD patients. Underlying mechanisms warrant further investigations.

Trial registration Registration number: NCT01660308 and NCT02620085

Keywords Fibroblast growth factor 23 · Graves' disease · Osteoporosis · Phosphorus

Introduction

Graves' disease (GD) is a thyroid disease with autoimmune basis resulting from genetic and environmental influences, featuring hyperthyroidism due to activating autoantibodies against the thyrotropin receptor [1]. In addition to thyroid dysfunction, other comorbidities including Graves' orbitopathy, cardiovascular diseases, psychiatric symptoms,

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and osteoporosis impose remarkable impacts on the quality of life [1–3]. Osteoporosis-associated fracture is one of the major complications of GD [4, 5], which is indicated by reduced bone mineral density (BMD) and accelerated bone turnover [6, 7]. Among various factors involved in the mineral homeostasis, fibroblast growth factor 23 (FGF23) is one of the most important phosphatonins regulating serum phosphorus and is significantly increased in subjects with untreated GD [6]. Several bone turnover markers were also elevated in hyperthyroid GD patients compared with the control group [6]. Serum FGF23 and phosphorus decreased after a 3-month treatment of GD (not all patients achieved euthyroidism at this time) [8]. A longitudinal study by Pantazi H. et al. disclosed that bone turnover makers including urinary N-telopeptides of collagen (NTX), serum alkaline phosphatase (ALP), and serum osteocalcin would keep decreasing during 1 year of antithyroid treatment even though thyroid hormones had been normalized within 10 weeks [9]. Some of these markers descended to reference ranges, while others did not. It remains unclear whether and when serum FGF23 and phosphorus will be normalized after euthyroidism is achieved in GD patients. The aim of this study is to unveil the differences of FGF23, phosphorus, and other major markers associated with bone and mineral metabolism between euthyroid GD and healthy population.

Patients and methods

Study subjects

This is a case-control study. Initially, participants with euthyroid GD (case) were enrolled from October 2011 to March 2012 at the National Taiwan University Hospital (NTUH). The ages of enrolled patients were between 20 and 85 years old, and diagnosis of euthyroid GD was established based on clinical manifestation, treatment course, and thyroid functional tests. Thyroid autoimmune profiles including thyrotropin-binding inhibitory immunoglobulin (TBII) and/or thyroid ultrasonography were performed for further confirmation if necessary [10]. Detailed diagnostic criteria for GD required at least one of the following conditions: (1) typical autoimmune characteristics on thyroid ultrasonography and a positive TBII; (2) Graves' orbitopathy (GO) with positive TBII and/or typical thyroid ultrasonography for GD; (3) history of antithyroid drug use for at least 1 year after documented hyperthyroidism with a positive TBII and/or typical thyroid ultrasonography of GD. Euthyroidism was defined as normal serum free thyroxine (fT_4) and thyrotropin (TSH) levels. Age- and sex-matched control subjects were derived from the Taiwan Lifestyle Study, a prospective community-based cohort study since 2006 [11–13]. Data of serum fT_4, TSH, TBII, anti-thyroperoxidase antibodies (anti-TPO), calcium, phosphorus, intact parathyroid hormone (iPTH), 25-hydroxyvitamin D (25(OH)D), and FGF23 were obtained. Serum C-terminal telopeptide of type 1 collagen (CTX) and N-terminal propeptides of type 1 collagen (PINP) were analyzed in only 23 euthyroid GD subjects and 13 control subjects because the blood samples of the other subjects were not left enough for such examination.

For the further study of tubular reabsorption of phosphate (TRP), we recruited another group of subjects, including 20 patients with euthyroid GD and 20 healthy subjects.

Informed consents were signed by all participants prior to the study, and ethical approval was obtained from the Ethics Committee of NTUH (protocol number 201105045RC, 201107013RC, and 201411013RINB).

Measurements of biochemical markers

Serum albumin, calcium, and phosphorus were analyzed by an automatic analyzer (AU5800 AU analyzer, Beckman Coulter, Inc., California, USA). Another automatic analyzer (ARCHITECT i2000SR Immunoassay analyzer, Abbott Co., Ltd., Illinois, USA) was used to test serum fT4, TSH, anti-TPO, iPTH, and 25(OH)D. Reference ranges of fT4 and TSH were 0.6–1.75 ng/dl and 0.1–4.5 μIU/ml respectively. Reference range of serum iPTH, Ca, and P was 11–62 pg/ml, 8.9–10.3 mg/dl, and 2.4–4.7 mg/dl, respectively. A 25(OH)D equal to or less than 24 ng/dL was defined as deficiency, whereas 25(OH)D between 25 and 80 ng/dL was defined as normal. The lowest detectable limit of anti-TPO was 0.3 IU/mL. TRP (%) was calculated using the following formula: $100 \times (1 - ((\text{urine phosphorus/urine creatinine}) \times (\text{serum creatinine/serum phosphate})))$. The normal range is between 85 and 95% when serum phosphorus is normal [14]. TBII was measured by TSH receptor autoantibody by RiaRSR™ TRAb CT kit (RSR, Co., Ltd., Lancaster, UK) [15].

Measurement of FGF23

Serum FGF23 was measured by a two-site ELISA assay (Kainos Laboratories, Inc., Tokyo, Japan) according to the manufacturer's instructions. Two specific murine monoclonal antibodies were bound to full-length FGF23. Capture antibody was immobilized onto the microtiter well. The other antibody was conjugated to horseradish peroxidase for detection by a spectrophotometric reader. The normal range for FGF23 is 8.2–54.3 pg/ml [8].

Measurements of bone turnover markers

Serum PINP was selected to represent bone formation, and serum CTX was examined as a marker of bone resorption [16]. PINP and CTX were analyzed by electrochemiluminescence immunoassay (ECLIA) using the immunoassay analyzer cobas e 411 (Roche Diagnostics, Indiana, USA). The normal ranges for

PINP are 15.13 to 58.59 ng/ml in pre-menopausal condition and 16.27 to 73.87 ng/ml after menopause. The normal ranges for CTX are 0.02 to 0.58 ng/ml and 0.1 to 1.0 ng/ml for pre- and post-menopausal conditions respectively.

Statistical analysis

Normally distributed continuous variables were presented as mean ± standard deviation (SD). Variables which were not normally distributed were presented as median (interquartile range), and statistical analyses were done after logarithmic transformation. Student's t test or χ^2 test were performed to examine statistical significance between different subgroups depending on the nature of variables. Pearson's correlation coefficient analysis was utilized to test the association between FGF23 and other markers related to mineral and bone metabolism. Linear regression model was used to analyze the relationship of FGF23 and phosphorus, using FGF23 as the dependent variable and serum phosphorus as the independent variable. Unadjusted and multivariate-adjusted regression coefficients were reported. A two-tailed p value below 0.05 was considered statistically significant. Stata/SE 14.0 for Windows (StataCorp LP, College Station, TX) was used for statistical analyses.

Results

First stage of the study

A total of 124 subjects were enrolled in the present study, with 62 patients of euthyroid GD and 62 participants in the control group. Average age was around 47–48 years old in both groups, with around 80% women in gender distribution. Median duration of euthyroidism from euthyroid status achievement to the enrollment of study in the GD group was 1.6 years with an interquartile range 0.7–2.8 years. Compared with the control group, subjects with euthyroid GD were associated with significantly elevated serum phosphorus, lower iPTH, and reduced 25(OH)D, while serum calcium levels revealed no difference (Table 1). As shown in Table 2, serum FGF23 was negatively associated with serum phosphorus in the euthyroid GD group but not in the control group. Other markers for mineral and bone metabolism such as calcium, 25(OH)D, iPTH, PINP, and CTX revealed no significant association with serum FGF23. Serum FGF23 was significantly increased in the euthyroid GD group as compared with that in the control group (Table 1). As shown in Table 3, serum FGF23 was negatively associated with serum phosphorus level in patients with euthyroid GD. The differences remained significant after adjusted for age, gender, calcium, iPTH, and 25(OH)D levels. In contrast, serum FGF23 of normal controls did not have a significant correlation with serum phosphorus in the linear regression analysis, using FGF23 as the dependent variable and serum phosphorus as the independent variable, whether before or after adjustment (Table 3).

We further analyzed the subgroups of hyperphosphatemia and normophosphatemia in patients with euthyroid GD. No subject had hypophosphatemia in our study. The results were shown in Table 4 and Fig. 1. The fT4 levels before management of hyperthyroidism were slightly higher in patients with hyperphosphatemia than in patients with normophosphatemia (Table 4). The relationship of serum FGF23 and phosphorus was shown in Fig. 1, and subgroups of normo- or hyperphosphatemia were demonstrated in different symbols. In the linear regression analysis using FGF23 as the dependent variable and serum phosphorus as the independent variable with the adjustment of age and gender, serum FGF23 and phosphorus were negatively correlated in the normophosphatemic group (regression coefficient = − 80.9, $p = 0.003$), and there was no correlation in the hyperphosphatemic group (regression coefficient = − 24.2, $p = 0.229$) (Fig. 1).

Second stage of the study

To further understand the TRP, we recruited another group of subjects, including 20 patients with euthyroid GD and 20 healthy subjects. The result was shown in Table 5. FGF23 was significantly higher, and TRP was significantly lower in patients with euthyroid GD than in normal controls ($p = 0.0403$ and 0.0015 respectively). In the linear regression analysis using FGF23 as the dependent variable and serum phosphorus as the independent variable, there was no significant correlation between serum FGF23 and phosphorus in patients with euthyroid GD (regression coefficient = − 15.5, $p = 0.382$) and in normal controls (regression coefficient = 10.6, $p = 0.407$).

Discussion

Our study showed that serum phosphorus and FGF23 were higher in patients with euthyroid GD than in healthy controls, even though these patients had achieved euthyroidism for a median of 1.6 years. To our knowledge, this is the first study which reveals the association between serum FGF23 and mineral metabolism, especially serum phosphorus, in GD patients who achieved euthyroidism.

Literatures showed that patients with hyperthyroidism due to GD have higher serum calcium, phosphorus, and FGF23 levels and lower 25(OH)D and iPTH levels than normal controls [6]. The underlying mechanism is supposed to be altered bone metabolism during thyrotoxicosis: higher osteoblast and osteoclast activities with a predominance of the latter [17]. The accelerated bone resorption results in mineral bone mobilization and efflux of calcium and phosphate from bone to systemic circulation [6, 17]. Hyperphosphatemia is also caused by the

Table 1 Clinical characteristics of study subjects in the first stage of study ($N = 124$)

N	Euthyroid GD 62	Control 62	*p*
Age (years)	47.4 ± 11.0	47.8 ± 10.9	0.9280
Gender (female:male)	50:12	49:13	0.823
TSH (μIU/ml)	1.14 (0.64–1.96)	1.10 (0.68–1.40)	0.7233
fT_4 (ng/dl)	0.99 (0.91–1.11)	1.01 (0.95–1.09)	0.3584
Anti-TPO (IU/ml)*	57.47 (2.7–399.87)	0.3 (0.3–0.3)	< 0.0001
TBII (%)†	15.9 (6.85–34.25)	0 (0–0)	< 0.0001
Corrected calcium (mg/dl)	9.13 ± 0.47	9.23 ± 0.29	0.2611
Phosphorus (mg/dl)	4.27 ± 0.77	3.84 ± 0.51	0.0022
Low:normal‡:high (%)	0:37:7 (0:84.1:15.9)	0:44:2 (0:95.7:4.4)	0.068
25(OH)D (ng/dl)	16.23 ± 5.46	23.31 ± 5.27	< 0.0001
iPTH (pg/ml)	15.27 ± 8.88	25.73 ± 11.37	< 0.0001
FGF23 (pg/ml)	59.10 (44.84–92.03)	45.52 (27.21–60.55)	0.0004
Low:normal§:high (%)	0:23:29 (0:44.2:55.8)	1:32:13 (2.2:67.6:28.2)	0.016
PINP (ng/ml) ‖	51.02 ± 19.04	54.12 ± 20.65	0.6552
CTX (ng/ml) ‖	0.21 ± 0.12	0.23 ± 0.09	0.6240

25(OH)D, 25-hydroxyvitamin D; *Anti-TPO*, antithyroid peroxidase antibody; *CTX*, C-terminal telopeptide of type 1 collagen; *fT4*, free thyroxine; *FGF23*, fibroblast growth factor 23; *iPTH*, intact parathyroid hormone; *NA*, not available; *PINP*, N-terminal propeptides of type 1 collagen; *TBII*, thyrotropin-binding inhibitory immunoglobulin; *TSH*, thyroid stimulating hormone

Data are presented as mean ± SD if the continuous variable is normally distributed, and as median (interquartile range) if not normally distributed

Corrected calcium was calculated as the following: serum calcium (mg/dl) + 0.8 × (4 − albumin (g/dl))

P values were calculated by *t* test for continuous variables and by chi-squared test for categorical variables

TSH, fT4, anti-TPO, TBII, and FGF23 were logarithmically transformed to become normally distributed for *t* test

*Reference range of anti-TPO < 5.61 IU/ml; lowest detection limit 0.3 IU/ml

†Reference range of TBII: negative if < 10%; positive if > 15%; borderline positive if 10–15%

‡Normal range of serum phosphorus level is 2.4–4.7 mg/dL

§Normal range of serum FGF23 level is 8.2–54.3 pg/mL

‖ Data of PINP and CTX were analyzed in 23 euthyroid GD subjects and 13 control subjects

Table 2 The correlation coefficients of serum fibroblast growth factor 23 (FGF23) and markers of bone and mineral metabolism in the euthyroid Graves' disease group and the control group. FGF23 was logarithmically transformed for statistical analyses.

	FGF23	
	Euthyroid Graves' disease group	Control group
Corrected calcium	0.0201	− 0.3939
Phosphorus	− 0.3298*	0.1620
25-OH vitamin D	− 0.2117	0.0218
iPTH	− 0.0247	− 0.0280
PINP	− 0.0315	0.2906
CTX	0.2569	0.2364

FGF23, fibroblast growth factor 23; *iPTH*, intact parathyroid hormone; *PINP*, N-terminal propeptides of type 1 collagen; *CTX*, C-terminal telopeptide of type 1 collagen

*Significant correlation with FGF23 ($p < 0.05$)

enhanced renal tubular reabsorption of phosphate by direct action of thyroid hormone [6, 18, 19]. Increased serum calcium suppresses parathyroid hormone (PTH). Suppressed PTH can also result in hyperphosphatemia, which in turn stimulates

Table 3 The relationship of serum fibroblast growth factor 23 (FGF23) and phosphorus in the euthyroid Graves' disease group and control group by linear regression model, using FGF23 as the dependent variable and serum phosphorus as the independent variable

	Euthyroid Graves' disease group	Control group
Crude		
Regression coefficient	− 31.6	8.3
p value	0.015	0.277
Adjusted for age, gender, calcium, parathyroid hormone, and 25-OH vitamin D		
Regression coefficient	− 34.9	16.311
p value	0.045	0.099

Table 4 Subgroup analysis by different serum phosphorus status in patients with euthyroid Graves' disease

N (%)	Normophosphatemia 37 (84.1%)	Hyperphosphatemia 7 (15.9%)	*p*
Age (year)	45.4 ± 11.7	49.5 ± 9.55	0.3767
Gender (female:male)	31:6	7:0	0.329
TSH (μIU/mL)	1.06 (0.43–1.96)	0.83 (0.34–1.84)	0.7987
fT_4 (ng/dL)	0.99 (0.93–1.06)	1.02 (0.97–1.17)	0.4318
Anti-TPO (IU/mL)	61.4 (1.48–406)	30.49 (1.72–946)	0.6614
TBII (%)	19.8 (9.1–41.8)	28.8 (10.1–39.3)	0.1521
Corrected calcium (mg/dL)	9.12 ± 0.47	9.23 ± 0.48	0.5589
Phosphorus (mg/dL)	4.04 ± 0.50	5.49 ± 0.80	< 0.0001
25(OH)D (ng/dL)	15.29 ± 4.39	21.1 ± 8.62	0.0098
iPTH (pg/mL)	16.23 ± 9.49	10.39 ± 4.01	0.1195
FGF23 (pg/mL)	56.95 (43.58–87.50)	65.34 (32.41–95.09)	0.6915
PINP (ng/mL)	51.57 ± 20.21	45.39 ± 23.52	0.6128
CTX (ng/mL)	0.19 ± 0.10	0.23 ± 0.14	0.5086
TSH before management (μIU/mL)	0.007 (0.004–0.15)	0.007 (0.004–0.018)	0.8709
fT4 before management (ng/dL)	2.11 (1.46–3.26)	4.8 (3.02–4.8)	0.0554
TBII before management (%)	56.5 (34.4–72.9)	67.3 (33.4–68.1)	0.7548

25(OH)D, 25-hydroxyvitamin D; *CTX*, C-terminal telopeptide of type 1 collagen; *fT4*, free thyroxine; *FGF23*, fibroblast growth factor 23; *iPTH*, intact parathyroid hormone; *PINP*, N-terminal propeptides of type 1 collagen; *TBII*, thyrotropin-binding inhibitory immunoglobulin; *TSH*, thyroid stimulating hormone

Data are presented as mean ± SD if the continuous variable is normally distributed, and as median (interquartile range) if not normally distributed

P values were calculated by *t* test for continuous variables and by chi-squared test for categorical variables

TSH, fT4, anti-TPO, TBII, and FGF23 were logarithmically transformed to become normally distributed for t-test

FGF23 production [6]. Elevated circulating inflammatory cytokines such as interleukins (IL) and tumor necrosis factors (TNF) in GD also upregulate osteocyte-derived expression of FGF23 [20–22]. Increased FGF23 facilitates renal excretion of phosphate, suppresses 1α-hydroxylation of 25(OH)D, decreases 1,25-dihydroxyvitamin D (1,25$(OH)_2$D), and leads to reduced serum calcium and phosphorus levels [6, 23]. The interaction between FGF23 and PTH could be complex [24]. In animal models and in vitro studies, FGF23 suppresses PTH synthesis through a calcineurin-sensitive pathway [25, 26], and FGF23 also activates FGF receptor 1 and its co-receptor, Klotho, on parathyroid cells to suppress PTH synthesis [25,

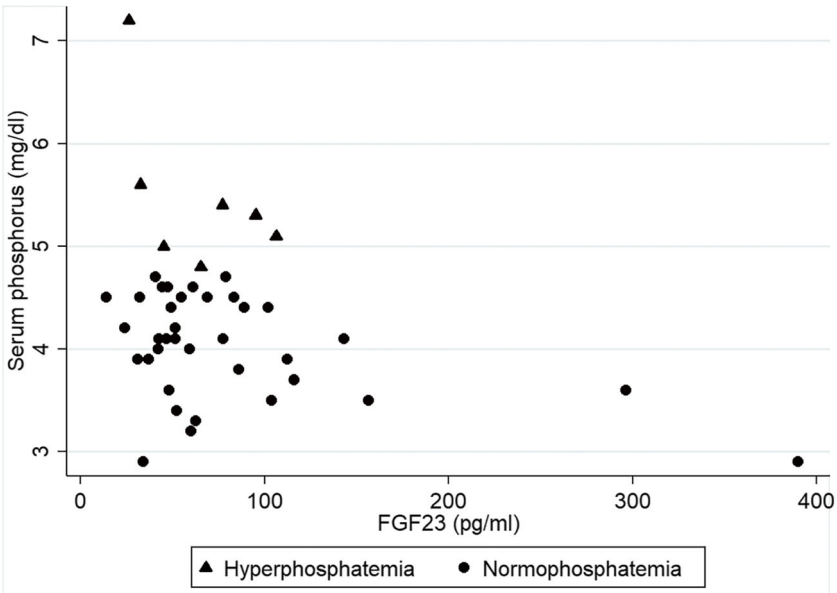

Fig. 1 The relationship between phosphorus and fibroblast growth factor 23 (FGF23) in patients with euthyroid Graves' disease

Table 5 Biochemical data of another 40 subjects in the second stage of the study for the observation of tubular reabsorption of phosphate (TRP)

N	Euthyroid GD 20	Control 20	P
Age (years)	48.7 ± 10.5	45.2 ± 12.4	0.3362
Gender (female:male)	16:4	16:4	
TSH (μIU/ml)	0.81 (0.43–1.78)	1.51 (1.21–2.15)	0.0034
fT4 (ng/dl)	1.02 (0.97–1.08)	0.88 (0.77–0.92)	0.0001
Corrected calcium (mg/dl)	8.86 ± 0.30	8.96 ± 0.20	0.2400
Phosphorus (mg/dl)	3.97 ± 0.41	4.08 ± 0.43	0.3939
25(OH)D (ng/dl)	28.00 ± 9.96	25.47 ± 10.86	0.4484
iPTH (pg/ml)	27.35 ± 10.60	28.46 ± 8.98	0.7216
FGF23 (pg/ml)	63.63 (56.45–83.52)	52.84 (44.39–68.25)	0.0403
PINP (ng/ml)	51.91 ± 16.77	45.9 ± 16.0	0.2628
CTX (ng/ml)	0.36 ± 0.17	0.32 ± 0.15	0.4919
TRP (%)	87.4 ± 4.4	91.7 ± 3.4	0.0015

25(OH)D, 25-hydroxyvitamin D; *CTX*, C-terminal telopeptide of type 1 collagen; *fT4*, free thyroxine; *FGF23*, fibroblast growth factor 23; *iPTH*, intact parathyroid hormone; *PINP*, N-terminal propeptides of type 1 collagen; *TSH*, thyroid stimulating hormone

Data are presented as mean ± SD if the continuous variable is normally distributed, and as median (interquartile range) if not normally distributed

P values were calculated by *t* test for continuous variables and by chi-squared test for categorical variables

TSH, fT4, and FGF23 were logarithmically transformed to become normally distributed for t-test

27]. But this may not be true in human physiology. For example, patients with tumor-induced osteomalacia often have a high serum FGF23 level and a normal or elevated iPTH level [24]. This indicates that FGF23 may not suppress PTH secretion in human. Despite the controversial findings in animal and human studies, PTH and vitamin D are documented to be lower in patients with GD and hyperthyroidism than in normal population [6]. Suppressed PTH would decrease serum calcium and increase serum phosphorus levels. Decreased $1,25(OH)_2D$ would promote the synthesis and secretion of PTH [23]. Lower 25(OH)D was observed in untreated GD patients in some studies [28], whereas conflicting evidence exists because of variable dietary vitamin D intake, different extent of sun exposure, seasonal variations or altered binding affinity with vitamin D–binding protein [6, 25]. It is postulated that patients with GD suffered from heat intolerance in early hyperthyroid status, and they tended to avoid sun exposure with the consequence of a lower 25(OH)D level.

These complicated interactions of hormones affect the mineral metabolism during thyrotoxicosis, and the biochemical profiles vary depending on the timing of blood sampling during the course of GD treatment. Consequently, the findings of normocalcemia, elevated serum phosphorus, increased serum FGF23, and decreased iPTH in subjects with euthyroid GD in our study represented only one of the variable aspects of the dynamic interplay of bone metabolism during the treatment of GD. We proposed the following mechanisms to explain the findings in our patients with euthyroid GD. First, homeostasis of the endocrine system from certain condition takes time and overshooting phenomenon on the way of returning to homeostasis exists in the endocrine system. One of the examples is the recovery from euthyroid sick syndrome: TSH levels in severely ill patients are reduced, followed by an increase in TSH above normal range during recovery [29]. This elevation in TSH persists until circulating fT4 and T3 levels return to normal. Follow-up observation generally reveals a normalization of TSH within 1–2 months [29]. In GD, elevated serum phosphorus and calcium due to high bone turnover during hyperthyroidism may persist for some period after euthyroid status is achieved. This can be supported by the fact that fT4 levels before management of hyperthyroidism are slightly higher in the hyperphosphatemic group than the normophosphatemic group in patients with euthyroid GD (Table 4). PTH decreases in response to the increased serum calcium. This decrease in PTH would precipitate an additional increase in serum phosphorus, which in turn stimulates FGF23 secretion. Serum calcium may be the first normalized mineral-associated substances during the treatment of GD. TRP levels at this time may be a comprehensive result of elevated FGF23, decreased iPTH, and probably the prolonged effect of hyperthyroidism on renal tubules. The above mechanisms appear to be the most plausible explanation for our results but remain to be proven as bone turnover markers of PINP and CTX in patients with euthyroid GD do not differ from those in normal controls. Other bone markers such as osteocalcin for bone formation and tartrate-resistant acid phosphatase or NTX for bone resorption should be evaluated [9, 30] . Second, the previously mentioned elevated circulating inflammatory cytokines such as IL and TNF are significantly higher in untreated GD patients than normal controls, which

would decrease after treatment of antithyroid medications, and do not totally return to normal even after euthyroid status reached for 6 months [22]. These cytokines may keep on stimulating FGF23 expression in patients with euthyroid GD. Increased FGF23 stimulated by persistent elevated cytokines in GD may also decrease TRP and result in its negative correlation with serum phosphorus. However, there was no euthyroid GD patient with hypophosphatemia and concomitant elevated FGF23 in our study, indicating that primary FGF23-mediated alteration in phosphorus should not be prominent in the whole picture.

Interestingly, serum FGF23, as a phosphatonin to enhance renal phosphorus excretion, was negatively associated with serum phosphorus in subjects with euthyroid GD in the present study (Tables 2 and 3, Fig. 1). The negative association remained significant after multivariate adjustments. Park S.E. et al. demonstrated a positive correlation between serum FGF23 and phosphorus in a cohort composed of both hyperthyroid GD and control participants (correlation coefficient = 0.457, $p < 0.05$) [6], but subgroup analyses were not performed to see the difference of association between hyperthyroid GD and control groups. Another study by Yamashita H. et al. disclosed that serum FGF23 and phosphorus kept decreasing during the 3-month treatment of hyperthyroidism, and serum FGF23 was positively correlated with phosphorus at this time [8]. However, above data were obtained during hyperthyroidism or during antithyroid treatments for only 3 months, and there were limited investigations focused on mineral metabolism in euthyroid GD patients in the literature, especially data in long-term cohorts. In Table 2, we demonstrated a negative association between serum FGF23 and phosphorus in the euthyroid GD group (correlation coefficient = − 0.3298, $p < 0.05$), but no significant association was seen in the control group. It is possible that the overwhelmingly elevated serum FGF23 in the early stage of GD fails to decrease in time as euthyroidism attains. The circulating inflammatory cytokines persistently elevate even after 6-month euthyroid status and keep on stimulating FGF23 expression and decreasing the reabsorption of phosphate in renal tubules [22]. This is supported by the higher FGF23 and lower TRP in the euthyroid GD group shown in Table 5. As a result, serum FGF23 could be negatively correlated with serum phosphorus in the initial phase of euthyroidism. Further longitudinal follow-up and investigations are warranted to prove all the above hypotheses.

Osteoporosis and its associated fractures are one of the major complications of GD [4, 5]. Reduced BMD and increased fracture risks were observed in untreated hyperthyroidism [7]. In addition, studies of bone turnover markers in hyperthyroid subjects are emerging. However, there is no defined consensus to conclude which marker is superior to the others in the evaluation of clinical osteoporosis [16]. One cohort study by Park S.E. et al. disclosed increased serum osteocalcin and CTX in hyperthyroid GD subjects [6], which is consistent with previous studies showing increased bone formation and resorption markers in the literature [4]. We examined serum PINP and CTX in selected subjects, and there was no significant difference between euthyroid GD and control groups. This could contribute to the limited case numbers, or alternative measurements are required to prove the differences. It is also possible that serum phosphorus, FGF23, iPTH, and 25(OH)D are more sensitive markers to detect abnormality of bone metabolism in GD patients than classic bone turnover markers.

Although there are finite studies exploring abnormal bone metabolism in euthyroid GD populations, one of the major differences between subjects with and without GD is the presence of autoantibodies against the thyrotropin receptors. Ercolano M.A. et al. reported a negative association between TSH receptor antibodies and BMD in euthyroid postmenopausal women with GD [31]. Another study compared the difference between stimulating and blocking activity of anti-thyrotropin-receptor antibodies on bone metabolism, which indicated that the presence of TSH receptor blocking antibodies was shown to prevent accelerated bone turnovers as compared with those who had stimulating TSH receptor antibodies in patients with GD [4]. In our study, there was a trend of higher TBII level in the hyperphosphatemic group than in the normophosphatemic group of patients with euthyroid GD ($p = 0.1521$) (Table 4).

There are some limitations of this study. First, this is a cross-sectional study, in which longitudinal association and causality of these biochemical markers could not be established. First, serum phosphorus, FGF23 and TRP were not checked at the time when hyperthyroidism was diagnosed. Second, TRP was not checked in the initial study of the 126 patients. Although TRP was checked in the second stage of the study, the case number was small. Third, we included only Han Chinese as a single ethnic group, and therefore racial differences could not be compared. Last but not least, the underlying mechanism are not explored in this epidemiological study.

In conclusion, the present study has demonstrated that subjects with GD had abnormal bone and mineral metabolism even after euthyroidism had been achieved for a long time. Subjects with euthyroid GD had higher serum phosphorus and FGF23 levels, and lower serum iPTH and 25(OH)D levels. The most plausible mechanism is that persistently elevated serum phosphorus due to high bone turnover stimulates FGF23 secretion in patients with euthyroid GD. Another finding is that serum FGF23 was negatively correlated with serum phosphorus in patients with euthyroid GD. Further investigations are warranted to elucidate longitudinal association and underlying mechanisms.

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Compliance with ethical standards

Informed consents were signed by all participants prior to the study, and ethical approval was obtained from the Ethics Committee of NTUH (protocol number 201105045RC, 201107013RC, and 201411013RINB).

Conflicts of interest None.

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