Association of Decreased Muscle Mass with Reduced Bone Mineral Density in Patients with Graves' disease

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Abstract

Background

The bone mineral density (BMD) did not increase significantly after the normalization of serum thyroid hormone levels. Studies on the effect of muscle mass on BMD in patients with Graves' disease are scarce. This study aimed to determine the association of decreased muscle mass with reduced bone mineral density in patients with Graves' disease.

Methods

A total of 758 patients with Graves' at diagnosis (mean age 41.2 years) were enrolled for a cross-sectional study; of these, 287 patients were enrolled for a cohort study with a median follow-up of 24 months. Meanwhile, 1164 age- and sex-matched healthy controls participants were recruited. All participants underwent dual-energy x-ray absorptiometry and muscle mass index (ASMI) measurements. The changes in ASMI and BMD were calculated from the measurements made at a gap of 2 years.

Results

Compared with healthy controls participants, the BMD was still significantly lower after normalizing serum thyroid hormone levels (1.131 ± 0.268 vs. 1.07 ± 0.133 , $p < 0.05$). ASMI was positively related to BMD in patients with Graves' disease (lumbar BMD, $r = 0.210$; femoral neck BMD, $r = 0.259$; hip BMD, $r = 0.235$; $P < 0.001$) and this relationship still existed after successful anti-thyroid therapy (lumbar BMD, $r = 0.169$; femoral neck BMD, $r = 0.281$; hip BMD, $r = 0.394$; $P < 0.001$). Low muscle mass was associated with low BMD (OR, 1.426; 95% CI, 1.019–1.994). Moreover, improving the muscle mass led to changes in the bone mass of the femoral neck (OR, 0.420; 95% CI, 0.194–0.911) and hip (OR, 0.217; 95% CI, 0.092–0.511) during the follow-up period. However, this phenomenon was not observed in lumbar, and bone turnover markers.

Conclusions

The recovery of bone mass might be related to the recovery of muscle mass. Improving muscle mass might bring about changes in the bone mass of the femoral neck and hip. A site-related discrepancy was also observed. Patients with Graves' disease should be helped in recovering muscle mass while administering anti-thyroid therapy.

1. Introduction

Patients with Graves' disease often exhibit various extrathyroidal manifestations [1], such as bone disease. Due to an excess of thyroid hormones, bone turnover accelerates, increasing the risk of fractures and osteoporosis [2]. A meta-analysis revealed that bone mineral density (BMD) significantly decreases and fracture risk increases in untreated hyperthyroidism [3]. A previous study showed that 40% of postmenopausal women with Graves' disease had severe osteoporosis, and 35% of them had an osteoporotic vertebral

fractures, independent of age[4]. Another study also demonstrated that a prior history of hyperthyroidism was independently associated with a fracture [5]. Euthyroidism would be beneficial to bone health and lead to good long-term outcomes[6]. However, the BMD was still lower after the normalization of serum thyroid hormone levels [7]. Hence, more studies are needed to detect potential factors associated with bone disease in patients with hyperthyroidism.

A decline in muscle mass and strength was found to be associated with overt thyroid disease[8]. With the successful treatment of hyperthyroidism, patients achieve euthyroidism. However, patients get a restoration of weight, particularly fat mass rather than muscle mass and bone mass, through normalization of the thyroid hormone levels [9], implying that muscle and bone tissues have a close developmental and functional relationship. Brennan et al. demonstrated that patients with hyperthyroidism had lower muscle strength and smaller midthigh cross-sectional area compared with individuals with euthyroid function [10]. However, they make no further reference to muscle mass or acknowledge that there may possibly be a correlation of muscle mass and BMD in Graves' patients. Graves' disease is the most common cause of hyperthyroidism. Little is known about the associations of skeletal muscle mass with bone mass in patients with Graves' disease besides thyroid hormone levels.

Some questions needed to be clarified: Is muscle mass related to bone health in patients with Graves' disease? Does this relationship exist after the successful treatment of hyperthyroidism? Does improving muscle mass bring about changes in bone mass? With these considerations in mind, the present study was performed to investigate the association between muscle mass and BMD in patients with Graves' disease before and after treatment. Furthermore, the association between 2-year changes in muscle mass and BMD was evaluated.

2. Materials And Methods

2.1 Study design

A facility-based cross-sectional study was performed to evaluate the association between muscle mass and BMD, while a cohort study was conducted to investigate the association between 2-year changes in muscle mass and BMD in the First Affiliated Hospital of Fujian Medical University. The approval of an ethics committee was obtained from the First Affiliated Hospital of Fujian Medical University, MRCTA, ECFAH of FMU [2017]131, and written informed consent was obtained from the patients.

2.2 Sample size calculation

The sample size was calculated using the Power Analysis and Sample Size software, version 11.0. The proportion of low muscle mass was assumed to be 65% for the low bone mass group (the case) and 50% for the normal-bone mass group (the control) based on the pilot study. Using the basic assumption of 80% power ($Z\beta = 0.84$) and 95% confidence interval (CI) ($Z\alpha/2 = 1.96$), the sample size was calculated as 208. Assuming that the data might be missing, 15% of data were added for compensation. As a result, the optimal sample sizes of the healthy control, Graves' at diagnosis, and Graves' after 2 years of therapy were preferred to be more than 245, respectively.

2.3 Inclusion criteria

(1) Patients with Graves' disease: All participants who met the US Thyroid Association 2016 Guidelines diagnostic criteria for Grave' disease and first diagnosis were included in this study [1].

(2) All patients received standard methimazole treatment prescribed by experienced endocrinologists. The patients with Graves' disease who were included follow-up study should have normal thyroid function.

(3) Participants with no Graves' disease: These included volunteers from community, company, enterprises, or public institutions of the Fuzhou area, China, for health examination, who had no Graves' disease.

2.4 Exclusion criteria

(1) Refusal to participate in the study.

(2) Patients with thyroidectomy, drug-induced hyperthyroidism, secondary or central thyrotoxicosis, extrathyroidal hyperthyroidism, hyperthyroidism crisis, or a history of failed anti-thyroid therapy.

(3) Patients with other causes of sarcopenia or bone loss, including poisoning and drug addiction[11].

(4) Intake of medications that might affect skeletal muscle functions or size, or long-term use of anti-inflammatory drugs or hormones[12].

(5) Patients with a history of spine or hip fracture, severe hepatic, heart disease and/or renal disease, alcoholism [13], hyperparathyroidism, and recent infections.

(6) Concomitant muscle, joint, or other conditions unrelated to the thyroid condition, limiting the mobility or the ability to accurately assess muscle mass.

(7) Athletes or pregnancy.

2.5 Sampling

Simple random sampling was employed to recruit study participants. Participants were selected by systematically recruiting every tenth patient from the outpatients between September 2012 and January 2018. The sample size was restricted to adults willing to participate so as to ensure research implementation. Of the initial 840 participants assessed for eligibility and employed at the time of the survey, only 327 were enrolled for the follow-up study. Several individuals had no dual-energy x-ray absorptiometry (DEXA) or medical history, or declined as personal preference. Therefore, these participants were not included in the analyses, leading to the final sample size of 758 in the Graves' at diagnosis, and 287 in Graves' after 2 years of therapy. Further, 1250 age- and sex-matched participants with no Graves' disease were recruited from the medical examination center. Finally, a sample size of 1164 was used in the analysis after various reasons for dropout. The detail of the subjects' recruitment is shown in Fig. 1.

2.6 Data collection

The patients' health records and history of the disease, intake of drugs including anti-thyroid therapy, smoking history [14, 15], drinking history [14, 15], exercising history [14, 15], and vegetable intake [16, 17] was obtained. Body height and weight were measured with the patients in light clothes and without shoes standing on the

RGZ-120-RT scale. Standardized measurements of systolic blood pressure (SBP) and diastolic blood pressure were conducted after a rest for 15 min. The body mass index (BMI) was calculated by dividing the weight by the squared height (kg/m²).

2.7 Clinical and biochemical measurements

All patients were fasted for more than 8 h, and blood was subsequently collected. The levels of glycated hemoglobin (HbA1c) (Variant II glycated hemoglobin analyzer, Bio-Rad, high-performance liquid chromatography), fasting plasma glucose (FPG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) (ADVIA 2400 automatic biochemical analyzer, Siemens, Germany), and serum concentrations of procollagen-type-1-N-terminal-propeptide (P1NP), and β -isomerized C-terminal cross-linked telopeptide (β -CTX), 25-hydroxyvitamin D [25(OH)D] were determined. The levels of thyroid hormones and autoantibodies were measured using a chemiluminescence immunoassay method: thyrotropin receptor antibody (TRAB) (0–1.75 IU/L), free triiodothyronine (FT3) (3.5–6.59 pmol/L), free thyroxine (FT4) (11.5–22.7 pmol/L), thyroid-stimulating hormone (TSH)(0.35–5.50 IU/mL), and thyroid peroxidase antibody (TPOAb) (0–60 IU/mL). The body composition, BMD and bone mineral content (BMC) were screened by DEXA (Lunar Prodigy scanner, GE Lunar Corporation, WI, USA). The body composition index was calculated using the following formula: ASMI = appendicular skeletal muscle mass (in kg)/height² (m²); body fat mass index (BFMI) = body fat mass (in kg)/height² (m²); body fat (%) = body fat mass (in kg)/body mass (in kg).

2.8 Definitions

Low bone density

Postmenopausal women and men aged more than 50 years should be considered to be of low bone density if they had a *T* score <−1 on DEXA after the exclusion of secondary causes of osteoporosis. Premenopausal women and men aged less than 50 years with a *Z* score $\leq$ −2.0 standard deviations (SDs) relative to the age-matched mean were also considered to be of low bone density [18, 19].

Low muscle mass

The ASMI cutoff point for the definition of low muscle mass was 7.23 kg/m² and 5.67 kg/m² for men and women, respectively, as initially proposed by the International Working Group on Sarcopenia[20]. According to the changes in muscle mass during the follow-up, the groups were classified as a decreased group (ASMI decreased to >3%), minor-change group (ASMI fluctuated from −3–3%), and increased group (ASMI increased to >3%) [21, 22].

BMD increment

The least significant change was defined as >2% [23]. Therefore, bone density increment (body, lumbar, femoral neck, and hip) was defined as (bone density at follow-up − primary bone density)/primary bone density >2%; others were defined as no change or decreased group. The aforementioned definition was used as the definition of BMC increment for reference.

CTX/P1NP increment

Bone resorption markers showed a circadian rhythm and decrease by 20–40% shortly after feeding, while bone formation markers had a little circadian rhythm (< 10%) [24]. Thus, CTX/P1NP increment was defined as (CTX/P1NP at follow-up – primary CTX/P1NP)/primary bone density > 40%/10%, respectively; others were defined as no change or decreased group.

2.9 Statistical analysis

All continuous variables were test for normality using Kolmogorov–Smirnov test. Statistical results were presented as mean ± standard deviation, median (interquartile range), or proportions. Analysis of Variance and Kruskal–Wallis tests were performed to determine differences for Gaussian variables and non-Gaussian variables. Linear regression analysis and Pearson correlation were used to analyze the correlation between bone density/content and ASMI. The multivariate logistic regression analysis of the relationship between muscle mass change and bone density/content increment was expressed as odds ratio (OR) and 95% CI after adjusting for multiple factors (detail in figure legend); the decreased group was taken as the reference group. Statistical tests were two sided; a *P* value less than 0.05 was considered to represent statistical significance. Statistical analyses were performed using the R software, version 3.5.1.

3. Results

3.1 Characteristics of the participants

The three groups were age and sex matched. The characteristics of participants are summarized in Table 1. As expected, patients with Graves' at diagnosis showed abnormal thyroid function, which was relieved after 2 years of therapy. TRAB and TPOAB also declined after therapy. SBP and FPG tended to be higher in patients with Graves' at diagnosis compared with healthy controls participants. The patients with Graves' at diagnosis had lower TC, TG, HDL, and LDL levels, which increased after therapy and did not differ significantly compared with healthy controls participants except for LDL. Otherwise, the patients with Graves' at diagnosis had lower BMI, body fat content, BFMI, and ASMI, and a higher rate of low muscle mass. BMI, body fat content, and BFMI increased and low muscle mass rate decreased after therapy. However, ASMI still differed significantly compared with healthy controls participants, suggesting that weight gain was mainly due to fat gain, and the muscle mass was not completely recovered in patients with Graves' disease during therapy. The patients with Graves' at diagnosis also showed higher levels of CTX and PINP and lower body bone content and BMD (body, lumbar spine, femoral neck, and hip), which reversed after therapy but still differed from those of healthy controls participants. The findings revealed that the recovery of bone mass might be related to the recovery of muscle mass. However, factors, such as smoking, drinking, exercising, and vegetable intake, did not differ significantly among the participants.

Table 1
Summary of the Participants characteristics

	Cross-sectional study			Follow-up study		
	Healthy controls (n = 1164)#	Graves' at diagnosis (n = 758)#		Graves' at diagnosis (n = 287)#	Graves' after 2 years of therapy (n = 287)	
Age(year)	41.1 ± 10.4	41.2 ± 12.2	0.745	40.0 ± 12.6		
Male, n (%)	366(31.4)	237(31.3)	0.935	85(29.6)		
SBP (mmHg)	114.7 ± 14.5	121.7 ± 15.8	< 0.001	120.9 ± 15.5	121.4 ± 17.5*	0.550
DBP (mmHg)	72.6 ± 10.3	73.1 ± 9.7	0.307	73.4 ± 9.9	76.5 ± 11*	< 0.001
BMI (kg/m2)	23.6 ± 3.3	20.9 ± 2.7	< 0.001	20.8 ± 2.7	23.2 ± 3.1	< 0.001
Smoking, n (%)						
Sometime, n (%)	130(11.2)	91(12)	0.813	26(9.1)	27(9.4)	0.317
Always, n (%)	85(7.3)	52(6.9)		27(9.4)	27(9.4)	
Drinking, n (%)	128(11)	95(12.5)	0.304	34(11.8)	34(11.8)	NS
Exercising, n (%)						
Sometime, n (%)	375(32.2)	210(27.7)	0.018	87 (30.3)	88(30.7)	0.223
Always, n (%)	296(25.4)	178(23.5)		71(24.7)	72(25.1)	
Vegetable, n (%)	729(62.6)	500(66)	0.137	194(67.6)	193(67.2)	NS
FT3(pmol/L)	5.04(4.54–5.58)	20.8(13.2–30.8)	< 0.001	17.965(11.995–29.955)	4.7(4.22–5.21)	< 0.001
FT4(pmol/L)	16.76(15.04–18.42)	55.77(36.14–80.27)	< 0.001	49.105(34.7–77.675)	15.83(13.97–17.74)	< 0.001
TSH(uIU/mL)	1.595(1.101–2.200)	0.005(0.0005–0.009)	< 0.001	0.005(0.0005–0.009)	1.905(0.377–4.060)	< 0.001
TRAb(IU/L)		11.87(6.06–21.23)		11.03(5.07–21.2)	1.61(0.95–3.83)	< 0.001
TPOAB(IU/mL)	28.00(14.97–34.93)	187.50(41.44–446.1)	< 0.001	149.7(28.37–374.35)	61.85(19.32–168.25) *	< 0.001
FPG (mmol/L)	5.04 ± 0.87	5.51 ± 0.91	< 0.001	5.51 ± 0.65	5.28 ± 0.66*	< 0.001

Post-hoc analysis: * compared with Healthy controls group, P < 0.05; #The three groups were age and sex matched.

	Cross-sectional study			Follow-up study		
HbA1c (%)	5.30 ± 0.41	5.54 ± 0.88	0.492	5.40 ± 0.48	5.23 ± 0.41	< 0.001
TC (mmol/L)	4.97 ± 1.02	3.66 ± 0.8	< 0.001	3.67 ± 0.8	4.98 ± 1.12	< 0.001
TG (mmol/L)	0.98(0.69–1.53)	0.90(0.70–1.23)	< 0.001	0.88(0.69–1.17)	1.01(0.76–1.48)	< 0.001
HDL (mmol/L)	1.61 ± 0.53	1.38 ± 0.39	< 0.001	1.44 ± 0.37	1.6 ± 0.48	< 0.001
LDL (mmol/L)	2.84 ± 0.88	1.94 ± 0.65	< 0.001	1.90 ± 0.63	3.05 ± 0.96*	< 0.001
25(OH)D(ng/ml)	26.31 ± 11.88	26.69 ± 9.48	0.471	26.76 ± 8.10	27.58 ± 9.75	0.274
Ca2+(mmol/l)	2.40 ± 0.18	2.45 ± 0.27	0.300	2.44 ± 0.27	2.41 ± 0.26	0.649
Body fat, n (%)	28.9 ± 6.9	27.6 ± 8.6	< 0.001	27.8 ± 8.6	29.2 ± 7.5	< 0.001
BFMI(kg/m2)	6.54 ± 2.11	5.87 ± 2.24	< 0.001	5.86 ± 2.24	6.65 ± 2.27	< 0.001
ASMI(kg/m2)	6.82 ± 1.22	6.05 ± 0.98	< 0.001	6.01 ± 0.93	6.66 ± 1.4*	< 0.001
CTX(ng/mL)	0.37(0.24–0.51)	1.19(0.89–1.56)	< 0.001	1.22(0.79–1.61)	0.56(0.31–0.73)*	< 0.001
P1NP(ng/mL)	41.39(30.79–53.98)	168.5(106.13–237.23)	< 0.001	184.35(95.65–255.975)	58.45(39.13–93.59)*	< 0.001
Low muscle mass,n (%)	222(19.1)	439(57.9)	< 0.001	173(60.3)	103(35.9)*	< 0.001
Body, Bone mineral content(g)	2450.7 ± 439.1	2229.6 ± 396.4	< 0.001	2245.1 ± 367.4	2352.7 ± 394.5*	< 0.001
Body ,Bone mineral density (g/m ²)	1.142 ± 0.115	1.072 ± 0.105	< 0.001	1.074 ± 0.097	1.109 ± 0.109*	< 0.001
Body,T score	0.507 ± 0.870	-0.170 ± 0.932	< 0.001	-0.342 ± 0.771	0.100 ± 0.856*	< 0.001
Body,Z score	0.596 ± 0.561	0.297 ± 1.144	< 0.001	0.047 ± 0.669	0.318 ± 0.755	0.035

Post-hoc analysis: * compared with Healthy controls group, P < 0.05; #The three groups were age and sex matched.

	Cross-sectional study			Follow-up study		
Lumbar ,Bone mineral density (g/m ²)	1.131 ± 0.268	1.016 ± 0.136	< 0.001	1.018 ± 0.122	1.07 ± 0.133*	< 0.001
Lumbar ,T score	-0.212 ± 1.325	-0.751 ± 1.404	< 0.001	-0.700 ± 1.292	-0.278 ± 1.425*	< 0.001
Lumbar, Z score	0.148 ± 1.24	-0.074 ± 1.206	< 0.001	-0.059 ± 1.142	0.205 ± 1.233	< 0.001
Femoral neck ,Bone mineral density (g/m ²)	1.017 ± 0.15	0.934 ± 0.145	< 0.001	0.947 ± 0.147	0.995 ± 0.131*	< 0.001
Femoral neck ,T score	0.464 ± 1.184	-0.683 ± 1.139	< 0.001	-0.627 ± 1.149	-0.246 ± 1.01*	< 0.001
Femoral neck ,Z score	0.639 ± 0.978	-0.072 ± 1.088	< 0.001	-0.04 ± 1.118	0.232 ± 0.934*	< 0.001
Hip ,Bone mineral density (g/m ²)	1.082 ± 0.143	0.894 ± 0.127	< 0.001	0.878 ± 0.121	0.932 ± 0.120*	< 0.001
Hip,T score	0.738 ± 1.077	-0.656 ± 0.969	< 0.001	-0.758 ± 0.932	-0.234 ± 1.128*	0.034
Hip,Z score	0.684 ± 0.847	-0.241 ± 0.887	< 0.001	-0.3 ± 0.918	-0.100 ± 0.889*	0.367
Low bone Mass,n (%)	135(11.6)	329(43.4)	< 0.001	113 (39.4)	84(29.3) *	0.002
Post-hoc analysis: * compared with Healthy controls group, P < 0.05; #The three groups were age and sex matched.						

3.2 Cross-sectional study

3.2.1 Association between ASMI and CTX, PINP, BMD, low muscle mass, and low bone density

Initially, the Pearson correlation and univariate linear regression analyses were performed to evaluate the association between ASMI and CTX, PINP, and BMD, separately for the patients with Graves' at diagnosis and Graves' after 2 years of therapy, as shown in Fig. 2. Furthermore, the multivariate regression analysis adjusted for age, BMI, sex, and other confounding factors showed that ASMI was positively associated with the body bone mineral content ($\beta = 84.52$, $P < 0.001$; $\beta = 169.53$, $P < 0.001$), body BMD ($\beta = 0.016$, $P < 0.001$; $\beta = 0.021$, $P < 0.001$), femoral neck BMD ($\beta = 0.033$, $P = 0.001$; $\beta = 0.023$, $P < 0.001$), and hip BMD ($\beta = 0.025$, $P = 0.003$; $\beta = 0.033$, $P < 0.001$) in the with Graves' at diagnosis and Graves' after 2 years of therapy. However, no significant

association was found between ASMI and CTX and PINP, as shown in Fig. 2. The multivariate logistic regression model also showed that the association between low muscle mass and low bone density remained significant (OR, 1.426; 95% CI, 1.019–1.994; $P = 0.038$), adjusting for age, BMI, sex, FT3, FT4, TSH, SBP, DBP, smoking history, and drinking history, exercising history, vegetable intake. Based on these findings, a nomogram was constructed using some significant variables (Fig. 3).

3.2.2 Simultaneous association of ASMI and FT4 with bone mineral content and BMD

Only FT4 was a significant variable among FT3, FT4, and TSH in the aforementioned multivariate logistic regression model. Thus, FT4 was used as a representative of thyroid function. Figure 4 shows the results for the simultaneous association of ASMI and FT4 with BMD in the patients with Graves' at diagnosis and Graves' after 2 years of therapy, adjusted for age, sex, and BMI. The standardized β -coefficient was used to directly compare the effect. In the adjusted models, a positive association was found between ASMI and BMC, and BMD (in the body, femoral neck, and hip) varied between 0.179 and 0.240 in the patients with Graves' at diagnosis. The associations still existed in patients with Graves' after 2 years of therapy. However, a negative association was found between FT4 and BMD of the femoral neck. BMD varied between -0.111 and -0.110 in the patients with Graves' at diagnosis and disappeared in patients with Graves' after 2 years of therapy. These standardized β -coefficients of ASMI on BMC and BMD were higher than those of FT4 on BMC and BMD, suggesting that the effect of thyroid hormones on bones was weaker than that on muscle mass. An excess of thyroid hormones is detrimental to both muscle mass and bones (body and femoral neck).

3.3 Follow-up study

Furthermore, a cohort study was performed on 287 returning patients with a median follow-up of 24 months to evaluate whether improved muscle mass led to changes in bone mass. As shown in Fig. 5, the body bone mineral content (OR, 0.281; 95% CI, 0.100–0.785) and BMD of the femoral neck (OR, 0.420; 95% CI, 0.194–0.911) and hip (OR, 0.217; 95% CI, 0.092–0.511) significantly improved in the ASMI increased group compared with the decreased group, after adjusting for age, sex, and other parameters. Even a minor change against age was beneficial for body bone mineral content. However, no association was found between body and lumbar BMD and bone turnover markers (PINP and CTX).

4. Discussion

This study was novel in assessing the possible associations between low muscle mass and BMD in patients with Graves' disease. It showed that muscle mass was positively related to bone health in patients with Graves' disease, and this relationship still existed after successful anti-thyroid therapy. Low muscle mass was associated with low BMD. Moreover, improving muscle mass led to changes in the bone mass of the femoral neck and hip during the follow-up period. However, this phenomenon was not observed in lumbar, and bone turnover markers.

Accumulating evidence shows that muscle and bone are anatomically and functionally closely connected [25]. A previous study on 95 type 1 diabetes children and adolescents from the tertiary center for pediatric diabetes

showed that type 1 diabetes influenced the musculoskeletal system in adolescence, and decreased muscle function contributed to osteoporosis reported in adult patients with diabetes [26]. The findings provided some hints about the association of muscle mass with bone health in younger patients. Another study showed that sarcopenia was closely associated with osteoporosis, and a low ASMI was a potential predictor of osteoporosis in patients with chronic liver disease and a median age of 65 (58–73) years [27]. A study on patients with chronic hepatitis C achieved similar results [28]. However, previous studies were mostly cross-sectional studies with a relatively small sample size. They did not focus on patients with Graves' disease. The deterioration in bone density due to an excess of thyroid hormones has been recognized for decades [2, 10, 29]. However, bone density has not increased completely as the normalization of serum thyroid hormone levels [7]. Studies on the effect of muscle mass on bone density in patients with Graves' disease still remain scarce. The present study suggested that the weight gain in patients with Graves' disease was mainly due to fat gain, and the muscle mass was not completely recovered in patients with Graves' disease during therapy. It was hypothesized that the delayed recovery of bone mass might be related to the recovery of muscle mass. Consequently, the BMD was not optimized if muscles did not grow in parallel with increased body weight. Yet, the present study found that the muscle mass was positively associated with BMD in patients with Graves' disease, and the relationship still existed after anti-thyroid therapy. Furthermore, the results were confirmed using a cohort. Cohort studies in this field are limited. A median follow-up of 2 years was used. A strong positive association was observed between the positive change in muscle mass and BMD, particularly in the femoral neck and hip. Several factors might account for the relationship. First, bone adapts its mass, architecture, and strength to changes in stress and strain induced by gravitational loading and muscle activity according to the biomechanical coupling theory [30, 31]. Second, muscles themselves secrete cytokines and growth factors that act paracrinally or endocrinally on bone metabolism. Several *in vitro* and *in vivo* studies demonstrated that many of these substances produced by muscles, such as insulin-like growth factor 1, osteonectin, and basic fibroblast growth factor, were involved in bone metabolism [32]. Ultimately, muscle and bone tissues have a close developmental and functional relationship [33]. However, they can also influence each other in the sense that a loss of muscle mass and function can lead to an activation of catabolic processes in bone and vice versa [34]. Surprisingly, this association was not observed in the case of bone turnover markers in both cross-sectional and cohort studies. It was speculated that bone turnover markers (serum CTX-I and PINP) were usually used to help assess the effect of nutrient interventions on bone in clinical trials [24]. Moreover, the bone turnover markers showed a circadian rhythm [35] and were affected by various environmental and physiological factors. Hence, evaluating the association between bone turnover markers and muscle mass was difficult, even with strict quality control. Participants unwilling to undergo retesting should also be mentioned.

Improving muscle mass led to changes in bone mass with site-specific differences. A robust association was observed only in the femoral neck and hip, but not in the lumbar region, in both cross-sectional and cohort studies. A plausible explanation for the beneficial effects of muscles on site-specific bones was that bones were more likely affected by a local interaction because the femur and hip were exposed to the largest muscle volume in the body. In addition, the femur is mainly composed of cortical bone, while the vertebra is mainly composed of trabecular bone [36]. Animal reports showed a decrease in the cortical thickness of the tibia and femur in mice with hindlimb unloading [37] and increased bone resorption along the cortex [38]. A similar trend was observed in another report [39]. The findings of this study also indicated that skeletal muscle and bone

losses were associated with overt thyroid disease, consistent with classic views[8]. However, no association was reported between the normal levels of thyroid hormones and bone and muscle mass after anti-thyroid therapy. This finding was contrary to the previous theory of the physiological level of thyroid hormones promoting bone and muscle growth. It was considered that patients with Graves' disease having a normal thyroid function after treatment were still not completely equivalent to healthy people[5]. Hence, different results were obtained.

This study proposed that improving muscle mass might lead to changes in bone mass. Unfortunately, it did not highlight ways to improve muscle mass. Some opinions were summarized for a reference. Resistance training was shown to increase the muscle cross-sectional area [40], but patients with sarcopenia might face potential difficulty with resistance exercises due to a decline in muscle mass. Protein and amino acid interventions, in combination with resistance training or additional supplements, had beneficial effects on muscle mass [21, 41]. Whole-body vibration therapy was found to significantly improve muscle mass [42].

This study had several strengths. First, the sample size of patients with Graves' disease was relatively large, and age- and sex-matched participants with no Graves' disease were recruited. Second, the cross-sectional study and cohort study were combined, thus enhancing the credibility of the results. Despite these strengths, the study had several potential limitations. First, the present study was performed in the hospital, leading to a potential selection bias. Second, the participants were younger, and hence the findings could not be generalized to assess the association between sarcopenia and osteoporosis. Thus, an interventional study, including a population with a broad age range, might help confirm whether an improvement in muscle mass can improve bone density or vice versa.

5. Conclusions

This study demonstrated that the BMD did not increase completely after normalizing serum thyroid hormone levels. The recovery of bone mass might be related to the recovery of muscle mass. A close association between low muscle mass and low BMD in patients with Graves' disease was observed. Improving muscle mass might lead to changes in the bone mass of the femoral neck and hip. Attention should be paid to help patients with Graves' disease recover muscle mass when administering anti-thyroid therapy.

Declarations

Author contributions

Yan S conducted the design of the study and reviewed the drafts. Zhang Y researched data and wrote manuscript. Li Y, Lin Y and Ke Y contributed to data collection. Huang L, Shen X and Zhao F co-wrote the final draft. All authors have read and approved the final manuscript

Conflicts of Interest

Yongze Zhang, Lingning Huang, Yuxi Lin, Ximei Shen, Fengying Zhao, Yuzhen Ke, and Sunjie Yan, declare that they have no conflict of interest. This work is not under active consideration for publication, has not been

accepted for publication, or has not been published, in full or in part. All co-authors adherence to ethical guidelines for publishing in the journal of cachexia, sarcopenia and muscle[43].

Statement of Human Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5).

Statement of Informed Consent

Informed consent was obtained from all patients for being included in the study. This study was approved by the Ethics Committee of the First Affiliated Hospital of Fujian Medical University, and the participants gave informed consent.

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A data availability statement

The dataset used to support the findings of this study are available from the corresponding author upon request.

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Figures

Figure 1

Detail of subjects' recruitment and follow-up, with reasons for dropout

Figure 2

Plot of ASMI and CTX, PINP, Bone mineral density, low muscle mass and low bone density (a) Linear regression (unadjusted) analysis and Pearson correlation were used to analyze the correlation between CTX, PINP, bone mineral density and muscle mass. (b) Linear regression(multivariate) adjusted for age, BMI, sex, FT3, FT4, TSH.

Figure 3

Nomogram for predicting the risk of low bone density in patients with Graves' disease Points for low muscle mass and other significant variables were obtained by calibrating with the point caliper and then combined to obtain a total score that could be calibrated with the risk of low bone density scale. As an example, using the points scale in Fig. 3, a man (12 points) aged 55 (61 points) with low muscle mass (13 points) has a FT4 level of 40pmol/l (11 points), and with a BMI of 20kg/m² (63 points), and therefore scores a total of 160. The lower scale of the nomogram shows that this score corresponds to a probability of low bone mass of about 0.73 (73 %).

Figure 4

The simultaneous association of ASMI and FT4 with bone mineral content and bone mineral density (a) Graves' at diagnosis (b) Graves' after 2 years of therapy The model has been adjusted for age, sex, BMI. The Standardized β -coefficient was used to directly compare the effect of ASMI and FT4 on BMC and BMD. *P < 0.05.

Figure 5

Associations between 2-year changes in muscle mass and bone mineral content, bone mineral density The model has been adjusted for age, BMI, sex, smoking history, and drinking history, exercising history, vegetable intake, changes of FT3, FT4, and changes of TSH. *P < 0.05.