

CLINICAL STUDY

Response of biochemical markers of bone turnover to oral glucose load in diseases that affect bone metabolism

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Abstract

Objective: Postprandial suppression of bone resorption is considered one of the main contributors in the circadian rhythm of bone turnover markers. The aim of this study was to investigate this physiological response of bone tissue in diseases that affect bone metabolism.

Patients and methods: In this study, 118 patients (45 hypothyroid, 40 hyperthyroid, and 33 β -thalassemic patients) and 78 healthy individuals matched for age and body mass index were included. An oral glucose test (75 g glucose) was performed after overnight fasting. Serum levels of procollagen type-I N-terminal propeptide (P1NP), β -C-terminal telopeptide of type I collagen (β -CTX), and osteocalcin were assayed at 0, 60, and 120 min.

Results: Baseline values of bone turnover markers were significantly elevated in hyperthyroid and β -thalassemic patients but not in hypothyroid patients compared with the control group. After oral glucose, the levels of β -CTX but not P1NP or osteocalcin were significantly suppressed in all groups (mean change from baseline is 46.9% for β -CTX, 7.9% for P1NP, and 8% for osteocalcin). The percentage change from baseline for β -CTX was significantly augmented in hypothyroidism (52 vs 42%, $P=0.009$).

Conclusion: The preservation or even augmentation of postprandial suppression of bone resorption in diseases that affect bone metabolism through distinct pathogenetic mechanisms suggests the importance of this physiological response to nutrients for the general homeostasis and functional integrity of the skeleton.

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Introduction

In the normal skeleton, bone remodeling shows a marked circadian pattern, with reduced bone resorption during daytime followed by nocturnal increase (1, 2). Recent studies identified nutrient supply as the initial event that triggers this postprandial suppression of bone resorption. The human skeleton, as a minerals' 'reservoir', participates in calcium homeostasis and supports hemopoiesis by stimulating or suppressing bone resorption depending on the availability of the nutrient in circulation. Key signals for the postprandial regulation of bone resorption seem to arise from the gastrointestinal tract and glucose-dependent insulinotropic peptide (GIP), and glucagon-like peptides (GLP-1 and -2) are considered to be the main candidates (3). Despite the significance of the nutrient-induced regulation of bone turnover in the general homeostasis and the functional integrity of the bone tissue, less is known about this postprandial adaptation of the skeleton in diseases that affect bone metabolism.

In a recent study by Chailurkit *et al.* (4), it was reported that the postprandial reduction of bone resorption was significantly attenuated in women with diabetes mellitus type 2.

Thyroid hormones (tri-iodothyronine (T_3) and thyroxine (T_4)) and the pituitary originated TSH exert a series of well-documented effects on skeletal development and growth, as well as, on the maintenance of adult bone mass and quality (5). Several studies have reported a marked relationship between thyroid status and bone mineral density (BMD) or fracture risk in healthy individuals as well as in patients with thyroid dysfunction (6–10).

At the cellular level, both the thyroid hormones and the TSH have been shown to play a direct role in bone metabolism, acting via specific receptors in bone cells (11–13).

Despite advanced knowledge regarding the molecular mechanisms involved in impaired bone metabolism in thyroid dysfunction, less is known about the functionality of the adaptive responses of the skeleton exposed to

low or high levels of thyroid hormones in physiological conditions.

β -thalassemia major is a hereditary hemoglobinopathy characterized by profound anemia due to a defect in the ability of erythroblasts to synthesize the β -chain of adult hemoglobin (14). Low bone mass and increased bone fragility in patients with β -thalassemia major lead to increased risk of fractures and represent an important cause of morbidity in this population (15, 16). The pathogenesis of thalassemia-induced bone disease is multifactorial and includes bone marrow expansion, endocrine dysfunction, and iron overload, as well as genetic susceptibility to attainment of low peak bone mass (17, 18). At tissue level, it has been demonstrated that there is an osteoblast dysfunction together with increased osteoclast activity leading to imbalanced bone turnover in favor of bone resorption (19–21).

This study was conducted to investigate the response of bone turnover markers after an oral glucose load in diseases such as hypothyroidism, hyperthyroidism, and β -thalassemia major that affect bone metabolism through pathogenetically distinct mechanisms.

Patients and methods

This study was approved by the local ethics committee of Aristotle University of Thessaloniki and was conducted according to the principles of the Helsinki Declaration II. Written informed consent was obtained from all participants.

A total of 120 consecutive patients with thyroid dysfunction that visited the outpatient Clinic of Endocrinology, Division of AHEPA University Hospital during the study period were initially screened for eligibility in the study. Of the 120 patients, 45 diagnosed with primary hypothyroidism (32 patients with Hashimoto thyroiditis, nine with multinodular goiter, and four with unidentified cause of hypothyroidism) and 40 diagnosed with hyperthyroidism (18 patients with toxic multinodular goiter, 12 with toxic adenoma, eight with Graves' disease, and two with diffuse toxic goiter and negative TSH-R antibodies) together with 45 healthy individuals matched for age and body mass index (BMI) were included in the final analysis. Inclusion criteria were diagnosis of primary hypothyroidism based on the laboratory results of increased TSH levels ($\text{TSH} > 8 \text{ mU/l}$) and diagnosis of hyperthyroidism based on the increased levels of free T_3 ($\text{FT}_3 > 8.1 \text{ pmol/l}$) and free T_4 ($\text{FT}_4 > 25 \text{ pmol/l}$), suppressed TSH levels ($\text{TSH} < 0.01 \text{ mU/l}$), and increased radioiodine uptake in thyroid scintigraphy. Patients with thyroid dysfunction due to subacute or drug-induced thyroiditis were excluded from the study. Additional exclusion criteria were previous use of anti-thyroid drugs or T_4 , concurrent diseases and medication that affect bone metabolism, postmenopausal women < 5 years after menopause, overt diabetes mellitus, impaired glucose tolerance (IGT)

or impaired fasting glucose, history of malabsorption, peptic ulcer, liver or kidney diseases, gastrointestinal operations, and inflammatory diseases of the gut. A thyroid ultrasound was performed for all patients.

Adult β -thalassemic patients were recruited from the Thalassemia Unit of AHEPA University Hospital. All patients were regularly transfused (every 1–3 weeks) and were subjected to frequent chelation programmes with subcutaneously administered desferoxamine (pumps) or/and oral iron chelators (deferiprone and deferasirox). Patients with endocrine dysfunction (i.e. hypo–hyperthyroidism, diabetes mellitus, hypoparathyroidism, and hypogonadism), liver or kidney disease, gastrointestinal problems, and vitamin D deficiency as well as patients with osteoporosis (assessed by DEXA of the hip and spine and based on the WHO criteria) or concurrent administration of medication that affects bone metabolism were excluded from the study. Finally, 33 patients with homozygous β -thalassemia major were enrolled. Due to significant differences in age and BMI of this group of patients compared with patients with thyroid diseases, 33 healthy volunteers matched for age and BMI, recruited from the medical school and the personnel of Aristotle University of Thessaloniki, were also included in the study.

Research protocol

After overnight fasting, all participants underwent a 75 g oral glucose tolerance test (OGTT) between 0730 and 0930 h. All samples were stored on ice until centrifugation at 4°C , after which serum was separated and stored at -20°C until analysis. Serum levels of procollagen type-I N-terminal propeptide (P1NP), β -C terminal telopeptide of type I collagen (β -CTX) and osteocalcin were assayed. For all participants, baseline levels of thyroid hormones, TSH, serum calcium, phosphate, PTH, blood urea nitrogen (BUN), creatinine, hematocrit, and hemoglobin were measured using standard laboratory methods. Plasma glucose levels at 0, 60, and 120 min were measured by a glucose oxidase colorimetric technique on an automated analyzer (Targa; Menarini, Florence, Italy).

Bone marker assays

Serum osteocalcin, P1NP, and β -CTX were measured using the Roche Elecsys 2010 platform (Roche Diagnostics), according to the manufacturer's instructions. Coefficients of variation are 5.5% for osteocalcin, 5.1% for β -CTX, and 1.9% for P1NP.

Statistical analyses

Results are presented as mean \pm S.E.M. The postprandial response of bone turnover is expressed as the percentage change from baseline at 2 h OGTT for β -CTX, P1NP,

and osteocalcin, as described previously (4). Differences between groups were assessed by two-tailed Student's *t*-test or the non-parametric Mann–Whitney *U* test for independent samples as appropriate. Paired sample *t*-test was used to assess differences between baseline values of bone markers and values at 2 h after oral glucose within each group. Correlations between the percentage of change for β -CTX at 2 h after oral glucose and baseline thyroid parameters FT₄, FT₃, and TSH in thyroid diseases or parameters of disease for β -thalassemic patients (ferritin and hemoglobin levels) were assessed by Pearson correlation coefficient (*r*). A *P* value <0.05 was considered statistically significant.

Results

Baseline characteristics of all the participants are shown in Tables 1 and 2.

Bone markers

Baseline values of serum P1NP, β -CTX and osteocalcin did not significantly differ between hypothyroid patients and the control group (P1NP: 41.45 ± 2.96 vs 42.7 ± 2.78 ng/ml, β -CTX: 333 ± 30 vs 372 ± 25 pg/ml, and Osteocalcin: 19.17 ± 1.12 vs 20.44 ± 1.89 ng/ml). On the contrary, patients with hyperthyroidism demonstrated significantly increased baseline values of bone turnover markers compared with the control group (877 ± 117 vs 372 ± 25 pg/ml for β -CTX, $P < 0.001$, 104.4 ± 13.8 vs 42.7 ± 2.7 ng/ml for P1NP, $P < 0.001$,

and 37.7 ± 3.9 vs 20.44 ± 1.9 ng/ml for osteocalcin, $P < 0.001$).

Baseline values of β -CTX and P1NP, but not osteocalcin, were significantly higher in the thalassemic patients compared with the control (450 ± 46 vs 340 ± 180 pg/ml, $P = 0.044$ for β -CTX, and 56.42 ± 7.08 vs 40.12 ± 2.80 ng/ml, $P = 0.039$ for P1NP).

After oral glucose, the levels of β -CTX were significantly suppressed in all groups (Figs 1 and 2), whereas no significant change was observed for osteocalcin and P1NP levels (Figs 1 and 2). The percentage suppression of β -CTX from baseline values after 2 h OGTT was significantly increased in hypothyroidism compared with the control group ($52.52\% \pm 2.6$ vs $43.21\% \pm 2.3$, $P = 0.009$; Fig. 3), and this difference remained robust when adjusted for baseline levels of β -CTX (adjusted means 52.48 vs 43.25% , $P = 0.010$). Despite high bone turnover status in patients with hyperthyroidism and β -thalassemia major, the percentage suppression of β -CTX after 2 h OGTT did not significantly differ compared with the respective control group ($42\% \pm 1.8$ vs $43.2\% \pm 2.3$, $P = 0.89$, in hyperthyroidism and $43.6\% \pm 0.02\%$ vs $45.3\% \pm 0.02\%$, $P = 0.684$ in β -thalassemia major) (Fig. 3).

Correlations

No significant correlation was found between the percentage reduction of β -CTX in the second hour and the baseline parameters of thyroid function ($r = 0.13$ for FT₃, $r = 0.212$ for FT₄, and $r = 0.189$ for

Table 1 Baseline laboratory values and epidemiologic characteristics of patients with hypothyroidism and hyperthyroidism.

	Control	Hypothyroidism	Hyperthyroidism	<i>P</i> value
Male	18	13	13	NS
Female	27	32	27	NS
Premenopausal women (%)	22 (81.5%)	25 (78%)	22 (81.5%)	NS
Total number	45	45	40	—
BMI (kg/cm ²)	27.1 ± 0.9	28 ± 1.56	26.79 ± 0.8	NS
Age (years)	49.8 ± 1.6	51.2 ± 1.56	50.2 ± 2.1	NS
FT ₃ (3.5–8.1 pmol/l)	5.6 ± 0.18	3.4 ± 0.2^a	22.11 ± 2.9^a	<0.001
FT ₄ (10.0–25.0 pmol/l)	16.18 ± 0.7	8.3 ± 0.5^a	46.9 ± 4.2^a	<0.001
TSH (0.3–4.0 mIU/l)	1.74 ± 0.15	22.3 ± 3.8^a	<0.01 ^a	<0.001
PTH (1.6–6.9 pmol/l)	4 ± 0.2	4.68 ± 0.2	3.6 ± 0.29	NS
25-OH vitamin D (30–120 nmol/l)	47 ± 1.3	41 ± 0.8	39 ± 1.1	NS
Serum calcium (8.2–10.6 mg/dl)	9.11 ± 0.07	9.35 ± 0.07	9.3 ± 0.08	NS
	(2.2 ± 0.1 mmol/l)	(2.3 ± 0.1 mmol/l)	(2.3 ± 0.2 mmol/l)	
Serum phosphate (2.7–4.5 mg/dl)	3.44 ± 0.17	3.8 ± 0.1	3.7 ± 0.09	NS
	(1.1 ± 0.05 mmol/l)	(1.2 ± 0.03 mmol/l)	(1.2 ± 0.02 mmol/l)	
BUN (10–50 mg/dl)	31 ± 1.47	35 ± 1.5	35.9 ± 1.6	NS
	(11 ± 0.5 mmol/l)	(12 ± 0.5 mmol/l)	(12.8 ± 4 mmol/l)	
Blood glucose (65–110 mg/dl)	92.9 ± 1.97	92.5 ± 2	89.5 ± 3.2	NS
	(5.1 ± 0.1 mmol/l)	(5 ± 0.1 mmol/l)	(4.9 ± 0.17 mmol/l)	
Serum creatinine (0.40–1.10 mg/dl)	0.77 ± 0.026	1.2 ± 0.3	0.9 ± 0.03	NS
	(58 ± 1.9 μ mol/l)	(91 ± 22 μ mol/l)	(68 ± 2.2 μ mol/l)	
Hct (37.0–47.0%)	40.6 ± 0.41	41.5 ± 0.7	39.28 ± 0.6	NS
Hb (12.0–16.0 g/dl)	13.6 ± 0.17	13.9 ± 0.25	13.38 ± 0.25	NS

NS, non-significant.

^aComparisons are with the control group.

Table 2 Baseline laboratory values and epidemiologic characteristics of patients with β -thalassemia major.

	Control	β -thalassemia major	P value
Male	10	13	
Female	23	20	
n	33	33	
BMI (kg/cm ²)	24.16 \pm 1.15	21.9 \pm 0.5	NS
Age	35.64 \pm 2	33.09 \pm 1.36	NS
TSH (0.3–4.0 mIU/l)	1.86 \pm 0.33	2.37 \pm 0.17	NS
PTH (1.6–6.9 pmol/l)	4.13 \pm 0.42	3.815 \pm 0.15	NS
25-OH vitamin D (30–120 nmol/l)	42 \pm 0.97	37 \pm 1.46	NS
Serum calcium (8.2–10.6 mg/dl)	9.11 \pm 0.07 (2.2 \pm 0.01 mmol/l)	9.35 \pm 0.07 (2.3 \pm 0.01 mmol/l)	NS
Serum phosphate (2.7–4.5 mg/dl)	3.21 \pm 0.34 (1 \pm 0.1 mmol/l)	3.86 \pm 0.14 (1.2 \pm 0.04 mmol/l)	NS
BUN (10–50 mg/dl)	31 \pm 1.32 (11 \pm 0.4 mmol/l)	28 \pm 1.2 (9 \pm 0.38 mmol/l)	NS
Blood glucose (65–110 mg/dl)	90 \pm 5.2 (4.9 \pm 0.2 mmol/l)	81.8 \pm 4.16 (4.5 \pm 0.2 mmol/l)	NS
Serum creatinine (0.40–1.10 mg/dl)	0.82 \pm 0.03 (62.5 \pm 0.01 μ mol/l)	0.77 \pm 0.4 (58.7 \pm 0.02 μ mol/l)	NS
Hct (37.0–47.0%)	41.55 \pm 1.9	29.2 \pm 0.36	<0.001
Hb (12.0–16.0 g/dl)	13.9 \pm 0.7	9.96 \pm 0.12	<0.001
Ferritin (30–400 ng/ml)	124.73 \pm 55.2 (280.2 \pm 124 pmol/l)	1269 \pm 119.3 (2851 \pm 268 pmol/l)	<0.001

NS, non-significant.

TSH levels, $P > 0.05$) in hypothyroidism or hyperthyroidism ($r = 0.122$ for FT₃, $r = 0.167$ for FT₄, and $r = 0.098$ for TSH levels, $P > 0.05$). In addition, we found no association between the percentage reduction of β -CTX after glucose load and ferritin levels ($r = 0.054$, $P = 0.788$) or hemoglobin levels ($r = 0.049$, $P = 0.654$) in thalassemic patients.

Discussion

In this study, we examined the response of bone turnover after an oral glucose load in patients with thyroid diseases and β -thalassemia major.

The role of nutrients in bone health and skeletal integrity is well established and disorders of nutrient supply due to abnormal intake or absorption, such as anorexia nervosa or inflammatory bowel diseases, respectively, have been consistently associated with low bone mass and increased fracture risk (22). From a pathophysiological point of view, stimulation of bone resorption during fasting allows the skeleton to act as a reservoir for rapid mobilization of mineral and buffer in the circulation, while during periods of adequate nutrient supply, bone remodeling adapts by suppressing bone resorption allowing the skeleton to shift the bone turnover balance in favor of bone formation (23).

In a recent study by Chailurkit *et al.* (4), this physiological skeletal response to glucose load was investigated in patients with diabetes mellitus type 2 and IGT. These researchers have demonstrated that the suppression of postprandial bone resorption is attenuated in patients with overt diabetes but not with IGT, suggesting an additional contributing factor in the deterioration of bone quality seen in diabetes (4).

In our study, in patients with hyperthyroidism and β -thalassemia major, despite the high bone turnover state observed at baseline, the postprandial reduction of

bone resorption remained unaltered, suggesting that the physiological responses of the skeleton to nutrients are conserved in these patients.

Hyperthyroidism is characterized by increased bone turnover and reduced BMD, followed by increased risk of fractures, which persists even after 5 years of treatment (9, 10, 24). Consistent with the published literature (9, 10), we also found increased bone turnover markers P1NP, osteocalcin, and β -CTX in hyperthyroid patients. Furthermore the significant suppression of β -CTX levels, but not P1NP or osteocalcin, after oral glucose, suggests that the exposure of the skeleton to thyroid hormone excess does not influence its physiological postprandial response. However, as information on the duration of hyperthyroidism in these patients is lacking, it remains unknown whether the time of exposure to increased levels of thyroid hormones would have any kind of influence in the postprandial adaptation of the hyperthyroid skeleton.

Bone disease in patients with β -thalassemia major is characterized by reduced bone formation rate accompanied by increased osteoclast activity. Several genetic factors, such as COL1A1 and VDR polymorphisms, and acquired factors due to primary disease itself (defective erythropoiesis and bone marrow expansion), iron overload, or toxicity due to iron chelation therapy have all been implicated in thalassemia-induced bone loss (17, 18). At molecular level, although osteoblast dysfunction is considered to be the main pathogenetic mechanism (19), recent evidence has demonstrated that anemia, through stimulation of erythropoietin and subsequent bone marrow expansion, also increases expression of RANKL and thus promotes activation of osteoclast activity (21, 25). In accordance with previous studies (20, 26) bone resorption marker β -CTX was found significantly increased at baseline compared with the control group. In addition, P1NP

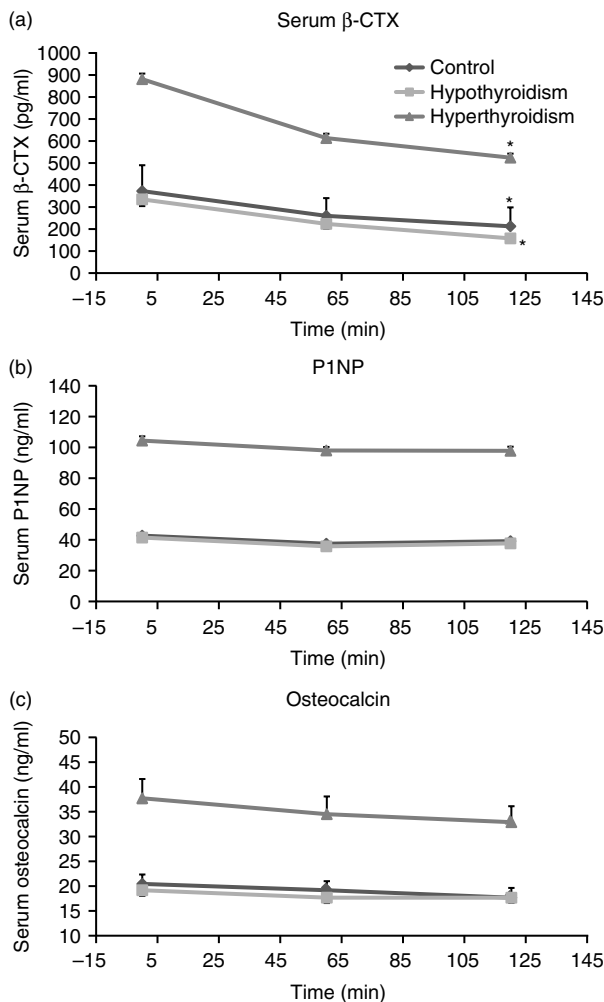


Figure 1 Effect of oral glucose on bone turnover markers in patients with thyroid diseases. Values are presented as mean \pm S.E.M., * $P < 0.001$ compared with baseline of the same group.

but not osteocalcin was also found increased in these patients, reflecting increased bone turnover. This would be considered to be in contrast with the study by Morabito *et al.* (25) who have shown decreased serum osteocalcin levels in patients with thalassemia major, suggesting decreased osteoblast activity. However, osteocalcin accounts for only a minor fraction of bone proteins, compared with collagen type 1, which constitutes 90% of bone matrix. Furthermore, osteocalcin gene expression is directly regulated by 1,25 vitamin D (27), which is usually decreased in thalassemic patients (28), and therefore the osteocalcin values in this population should be treated with caution. In our study, we have shown that despite the increased bone turnover rate, the postprandial response of bone resorption remained unaltered in β -thalassemic patients. Furthermore, the percentage reduction in β -CTX was not found to be associated with parameters of the disease in thalassemic patients such as ferritin levels, which is used as

an index for iron overload, or hemoglobin levels (degree of anemia).

Data of bone turnover markers in hypothyroid patients have presented inconclusive results reporting low or normal levels, although BMD is usually within normal limits (8, 29). In histomorphometry studies, however, the bone remodeling cycle was found significantly prolonged associated with decreased bone turnover rate (25, 30). In addition, large population studies have shown a two- to three-fold increase in fracture risk even after 10 years following initial diagnosis of hypothyroidism (8, 31).

At molecular level, *in vitro* and *in vivo* studies have demonstrated that deficient T_3 signaling through the TRa1 receptor in bone cells is the principal mediator of skeletal consequences of hypothyroidism independently of TSH levels (11–13).

In agreement with other studies, we have shown that baseline values of bone turnover markers did not differ

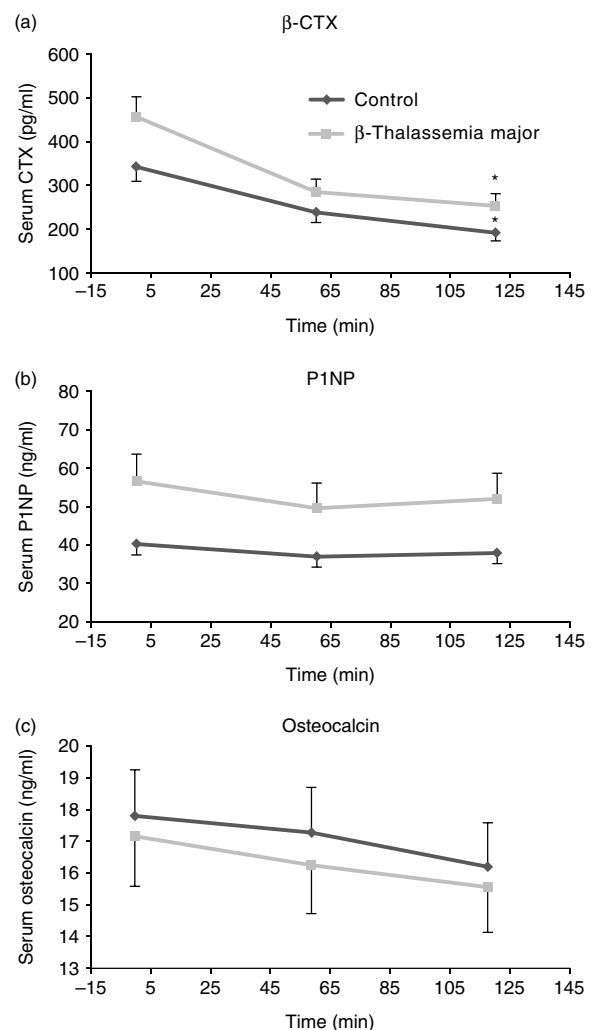


Figure 2 Effect of oral glucose on bone turnover markers in patients with β -thalassemia major. Values are presented as mean \pm S.E.M., * $P < 0.001$ compared with baseline of the same group.

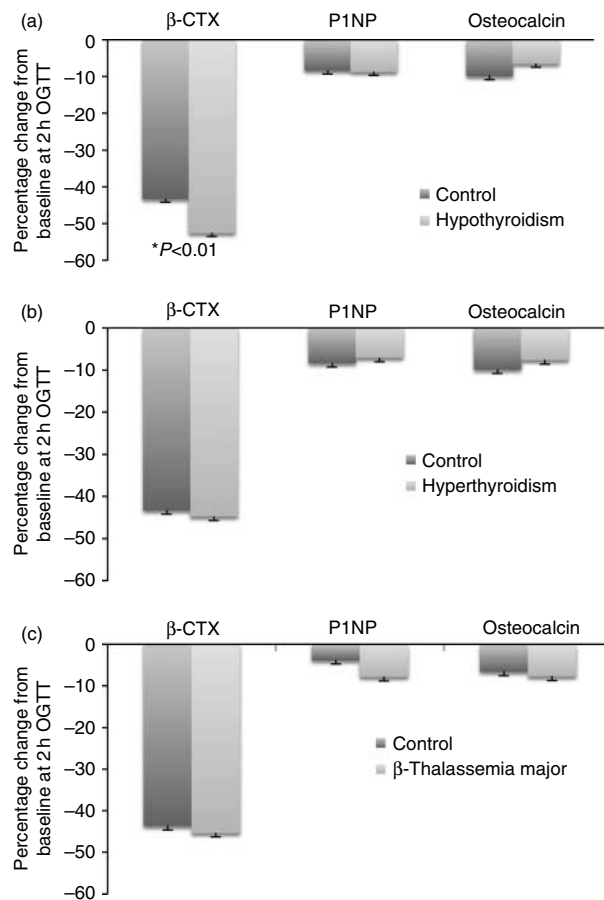


Figure 3 Percentage change from baseline at 2 h OGTT for bone turnover markers in patients with (a) hypothyroidism, (b) hyperthyroidism, and (c) β -thalassemia. Values are presented as mean \pm S.E.M., * $P < 0.001$ compared with control group.

between hypothyroid and healthy euthyroid patients. However, the postprandial suppression of bone resorption was significantly increased. The augmentation of this physiological response that benefits the skeleton in hypothyroidism, could in part compensate for the negative effect of T_3 deficiency in bone metabolism. This response, however, did not seem to be related to the severity of the disease, because no correlation was found between the percentage change from baseline and the thyroid function parameters.

It has been reported that the gut peptides GIP and GLPs are the most eligible candidates in mediating the postprandial reduction of bone resorption (3). Thyroid diseases affect the function of the gastrointestinal system causing decreased or increased motility and gastric emptying in hypothyroidism and hyperthyroidism respectively (32), which are usually resolved after restoration of the thyroid function. Older studies have investigated the secretion of GIP in hypo- and hyper-thyroidism showing no significant differences in GIP response to glucose load (33, 34), whereas there are

no studies regarding the GLP-1 and -2 secretion in these patients, or in patients with thalassemia major.

At this point, a reference should be made to the slight difference observed between our results and other studies regarding the percentage of change in β -CTX after oral glucose in healthy individuals (4.8). We found an $\sim 42\%$ decrease in β -CTX levels, whereas in the studies of Henriksen *et al.* (3) and Chailurkit *et al.* (4), this change was $\sim 50\%$. However, considering the fact that the levels of bone markers in the circulation present an acute reflection of the dynamics of bone metabolism, the use of age- and BMI-matched control group and the use of exactly the same conditions throughout the experiment should eliminate possible misinterpretations of our results.

In summary, our data demonstrate that despite impaired bone remodeling, the physiological event of postprandial reduction of bone resorption is preserved or even augmented in thyroid diseases and β -thalassemia major, supporting the importance of this phenomenon in the reservation of skeletal function and integrity. Despite intense research of the last years in the area of bone physiology, the factors that regulate the integration of energy homeostasis and bone metabolism are still far from being fully elucidated and future studies are warranted to shed light on the impact of the pancreatic and enteric hormones on bone physiology in health and disease.

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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