Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com

Association between thyroid hormone levels, the number of circulating osteoprogenitor cells, and bone mineral density in euthyroid postmenopausal women

Matteo Pirro, Maria Rosaria Manfredelli, Anna Maria Scarponi, Graziana Lupattelli, Francesco Bagaglia, Francesco Melis, Elmo Mannarino*

Unit of Internal Medicine, Angiology and Arteriosclerosis Diseases, Department of Clinical and Experimental Medicine, University of Perugia, Perugia, Italy

ARTICLE INFO

Article history:
Received 11 June 2011
Accepted 10 September 2011

ABSTRACT

In postmenopausal women, an association between reduced bone mineral density (BMD) and increased number of circulating osteoprogenitor cells (COPs) has been found. Although an increased thyroid function is associated with BMD, thyroid hormones stimulate osteoblast function in vitro. We investigated whether thyroid hormones within the reference range were correlated with the number of COPs and stimulate mineralization in vitro. The number of COPs, defined as CD34+/alkaline phosphatase (AP)+ or CD34+/osteocalcin (OCN)+ cells, was quantified by fluorescence-activated cell sorting (FACS) analysis in 150 euthyroid postmenopausal women. Participants underwent measurement of serum free thyroxine (FT4), thyroid-stimulating hormone levels, and femur BMD. CD34+ cells were isolated from healthy volunteers irrespective of AP or OCN expression, and the effect of triiodothyronine (0.5-10 pmol/L)) on their ability to form mineralized nodules in vitro was studied. The number of COPs was highest among women with high-normal FT4 levels (>1.09 ng/dL). The FT4 levels were correlated positively with circulating log-CD34+/AP+ ($r = 0.32$, $P < .001$) and log-CD34/OCN+ cells ($r = 0.36$, $P < .001$) and inversely with total femur BMD ($r = -0.17$, $P = .036$) but not with femoral neck BMD. In a multivariate analysis, the FT4 levels were positively correlated with the number of COPs, independent of age and BMD. The ability of CD34+ cells to form mineralized nodules increased after exposure from low up to high-normal triiodothyronine concentrations (P for trend = .003). Among euthyroid postmenopausal women, high-normal FT4 levels are correlated with an increased number of circulating immature osteoprogenitor cells and a very mild BMD reduction. Exposure of CD34+ cells to physiological triiodothyronine concentrations stimulates mineralization in vitro.

Author contributions: All authors have made substantial contributions to all of the following: (1) the conception and design of the study, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be submitted.

* *Corresponding author*. Hospital "Santa Maria della Misericordia," Piazzale Menghini, 1-06129, Perugia, Italy. Tel.: +39 75 5784024; fax: +39 75 5784022.

E-mail address: mpirro@unipg.it (M. Pirro).

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.09.006

1. Introduction

Alkaline phosphatase (AP)– and osteocalcin (OCN)-positive cells, also named *circulating osteoprogenitor cells* (COPs), have been found in the peripheral blood of adult human subjects [1,2]. These cells are able to promote in vitro and in vivo bone formation [1] and to contribute to osteogenesis in the early stages of fracture healing [3]. Strong evidence suggests that COPs are derived from bone marrow and are of hematopoietic origin [4]. Thus, other than bone-related proteins, COPs may also express the hematopoietic stem cell antigen CD34 [2,5]. The coexpression of CD34 and bone-related proteins AP and OCN has been proposed to identify a population of immature COPs [2,6].

Recently, we found that women with postmenopausal osteoporosis have an increased number of peripheral CD34+/OCN+ and CD34+/AP+ compared with nonosteoporotic postmenopausal women [6]; interestingly, reduced bone mineral density (BMD) was a significant independent predictor of the increased COP number [6]. Hence, we hypothesized that reduced BMD might represent a possible stimulus to increase COP numbers [6]. Whether additional factors, other than reduced BMD, contribute to the number of COPs is under investigation [4,7].

Thyroid hormones stimulate osteoblast activity both directly and indirectly via numerous growth factors and cytokines [8]. Triiodothyronine increases the expression of several bone-related genes in osteoblastic cells, such as OCN [9], AP [10], collagen type I [11], gelatinase B, and collagenase 3 [12], thus suggesting that thyroid hormones stimulate osteoblast differentiation [10,13]. However, thyroid hormones may also have a detrimental influence on mineralization. In rats, high triiodothyronine doses had a negative effect on osteogenic differentiation and reduced collagen synthesis in bone marrow stem cells [14]. Moreover, Britto et al [15] found that thyroid hormones can act on osteoblasts to indirectly stimulate osteoclastic bone resorption. The deleterious effects of thyroid hormones on in vitro mineralization by osteogenic cells is supported by in vivo studies demonstrating a significant association between hyperthyroid states and reduced BMD [16-20].

Hence, thyroid hormones may profoundly affect BMD in hyperthyroid states [16,17] and osteoblast activity in vitro [8-15] and also increase the number and the clonogenic potential of the hematopoietic progenitors [21]; thus, we investigated in postmenopausal women whether plasma thyroid hormone levels within a physiological range may influence BMD, the number of COPs expressing bone-related proteins and CD34, and the ability of CD34+ cells to form mineralized nodules in vitro.

2. Materials and methods

2.1. Study subjects

The study population consisted of 150 postmenopausal women independent in daily living activities and attending our Unit of Bone and Mineral Metabolism for screening for postmenopausal osteoporosis. Women were considered postmenopausal if they had not been menstruating for at least 1 year.

Exclusion criteria included a history of chronic diseases, such as thyroid, renal, hepatic, cardiac, and rheumatic diseases; current or prior use of drugs that could interfere with bone mass (ie, glucocorticoids, antiresorptive drugs, and hormonal replacement therapy); and a history of traumatic fractures. A trained interviewer gave each participant a questionnaire regarding age, ages related to menstrual history (menopause and menarche), smoking habits, familial history of hip fractures, personal history of fragility fractures, medical history, comorbid diseases, and use of medications. Information was also obtained by a review of medical records and laboratory data. The study was approved by the local Ethics Committee, and all participants gave their informed consent.

2.2. Clinical evaluation and BMD

All the determinations were made at the medical center at 8 AM, with a room temperature between 21°C and 23°C and after a 13-hour overnight fast. Height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively; subjects were wearing hospital gowns and had bare feet. Body mass index (BMI) was calculated as weight in kilograms divided by height squared in meters.

Areal BMD (grams per square centimeter; bone mineral content relative to the projection area) was measured by DXA (Hologic Discovery W; Hologic, Bedford, MA) at the proximal femur, with a coefficient of variation in our laboratory of 0.51%. The results for areal BMD were transformed to T scores, calculated as the difference between the actual measurement and the mean value of healthy sex-matched adult controls divided by their standard deviation. Self-reported or radiology-documented fragility fracture occurring at least 6 months before the study recruitment was also registered. Fragility fractures were defined as those that occurred without trauma or falling from a standing height or less.

2.3. Biochemical assays

Free triiodothyronine, free thyroxine (FT4), and thyroid-stimulating hormone levels were measured by a chemiluminescent immunoassay system (UniCel DxI 800; Beckman Coulter, Brea, CA). The bone specific isoenzyme of AP was measured by an immunoradiometric assay (Tandem R Ostase; Pantec, Torino, Italy). A radioimmunoassay was used to measure serum 25-OH-vitamin D (DiaSorin, Stillwater, MN). Serum C-terminal telopeptide of type I collagen (sCTX) was measured by enzyme-linked immunosorbent assay (Pantec).

2.4. Assay of circulating osteoprogenitor cells

The measurement of circulating osteoprogenitor cells was performed as previously described [6]. Mononuclear cells were isolated from platelet depleted peripheral venous blood by density centrifugation (Lymphoprep; Axis-Shield PoC, Oslo, Norway). The exclusion of nonviable mononuclear cells was performed by staining with 7-aminoactinomycin D (Beckman Coulter, Fullerton, CA). Freshly isolated mononuclear cells were incubated for 30 minutes at 4°C in the dark with a biotinylated antibody against human AP (R&D Systems, Minneapolis, MN), ECD-conjugated streptavidin (Beckman

Coulter), FITC-conjugated antibody against human CD34 (Beckman Coulter), Pc5-coniugated antibody against CD15 (Beckman Coulter), and PE-coniugated antibody against intracellular OCN (clone number 190125; R&D Systems) according to manufacturer's instructions. An anti-CD15 antibody was used to exclude contamination of isolated mononuclear cells with granulocytes. Isotype-identical antibodies at a concentration matched with specific antibodies served as controls (Beckman Coulter). All the antibodies were titrated to achieve working concentrations. After incubation, quantitative analysis was performed on a Coulter Epics XL measuring 100 000 cells per sample. Circulating osteoprogenitor cells were defined by negative staining for CD15, and either double positive staining for anti-CD34 and anti-AP or double positive staining for anti-CD34 and anti-OCN. The number of COPs was calculated by multiplying the frequency of fluorescent-positive events in the gate of lymphomonocytes by the total lymphomonocyte count. The COP count in 2 separate blood samples for each participant (subsample of 45 subjects) was highly reproducible ($r = 0.94$; $P < .001$).

3. In vitro mineralization by CD34+ cells

CD34+ cells isolated from healthy volunteers by miniMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) were amplified in cultures added with MethoCult (StemCell Technologies, Vancouver, Canada). The CD34+ cells obtained from amplification were cultured, irrespective of AP or OCN expression, in uncoated plastic culture plates with MesenCult Osteogenic Stimulatory Media (StemCell Technologies) and with increasing concentrations of triiodotyronine (Sigma-Aldrich, St Louis, MO) for 21 days, with the medium being changed every 3 days. The following triiodotyronine concentrations, titrated up to the high-normal physiological serum levels, were used: 0.5, 1.5, 2, 5, and 10 pmol/L. Subsequently, the OsteoImage Mineralization assay was performed to assess in vitro mineralization qualitatively by fluorescent microscopy (Nikon Eclipse TE2000, Nikon Instrumentation, Tokyo, Japan) using an F-ViewII FireWire camera (Soft Imaging System, Olympus Corp, Tokyo, Japan) and quantitatively using a plate reader (Tecan Infinite M200, Tecan Group Ltd., Mannedorf, Switzerland). The assay is based on the specific binding of the fluorescent OsteoImage Staining Reagent to the hydroxyapatite portion of mineralized bone nodules in osteoblast or osteogenic stem cell cultures. [22,23]. Captured images were processed and analyzed with the Cell^F View image software (Soft Imaging System, Olympus), and the results were expressed in relative fluorescence units (RFUs). The average results of 5 separate experiments are presented.

3.1. Statistical analysis

The SPSS statistical package, release 17.0 (SPSS, Chicago, IL) was used for all statistical analyses. Values are expressed as the mean ± SD. Analysis of variance and Kruskal-Wallis tests were used to compare the study variables between tertiles of FT4 levels (first tertile <0.82 ng/dL, second tertile 0.82-1.09 ng/dL, third tertile >1.09 ng/dL). Correlation analyses were performed using the Pearson and Spearman coefficients of correlations (r and ρ, respectively). A partial correlations procedure was computed to obtain partial correlation coefficients that describe the linear relationship between FT4 and additional variables while controlling the effect of confounders. Stepwise regression analysis was used to estimate prediction of FT4 (first model), BMD (second model), and COPs (third model) including the following independent variables: age, BMI, history of fragility fractures, BMD, 25-OH-vitamin D, bone AP, and CTX levels (first model); age, BMI, 25-OH-vitamin D, bone AP, CTX levels, and the log-transformed count of either CD34+/AP+ or CD34+/OCN+ cells (second model); and FT4, BMD, and history of fragility fractures with forcing of additional confounders (third model). Standardized coefficients were calculated as a measure for the relative predictive value. Repeated-measures analysis of variance was used to compare average RFUs. A statistical significance was assumed if a null hypothesis could be rejected at $P = .05$.

4. Results

The characteristics of 150 euthyroid postmenopausal women are provided in Table 1. On average, participants had a femoral neck T-score in the range of osteopenia. Furthermore, average levels of 25-OH-vitamin D and CTX appeared to be less than and slightly greater than the laboratory's reference ranges, respectively.

4.1. *Higher FT4 levels are predicted by low 25-OH-vitamin D levels*

Free thyroxine levels were significantly correlated with 25-OH-vitamin D ($\rho = –0.25$, $P = .002$). Stepwise regression analysis adjusted for potential confounders showed that 25-OH-vitamin D levels predicted FT4 levels (standardized $\beta = –0.27$, $P = .004$) independent of age, BMI, history of fragility fractures, femoral neck BMD, bone AP, and CTX levels.

Table 1 – Clinical and biological characteristics of 150 postmenopausal women

Age, y	65 ± 11
Age of menarche, y	13 ± 2
Age of menopause, y	48 ± 5
History of fragility fractures, %	34
BMI, kg/m^2	26.4 ± 4.6
Smokers, %	27
LDL cholesterol, mg/dL	137 ± 35
Triglycerides, mg/dL	103 (78-140)
FT4, ng/dL	1.02 ± 0.30
25-OH-vitamin D, ng/mL	14.1 (9.1-21.9)
Bone AP, μg/L	13.5 (10.5-16.3)
sCTX, ng/mL	0.78 (0.51-1.1)
Femoral neck BMD, g/cm^2	0.61 ± 0.13
Femoral neck T-score, SD	-2.05 ± 1.34
Total femur BMD, g/cm^2	0.77 ± 0.15
Proximal femur T-score, SD	-1.39 ± 1.26

Values are either mean ± SD or median and interquartile range for variables with a skewed distribution. LDL indicates low-density lipoprotein.

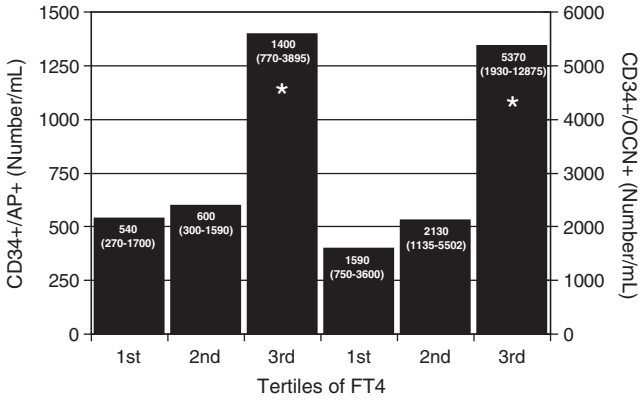

Fig. 1 – Number of circulating CD34+/AP+ and CD34+/OCN+ in the 3 tertiles of FT4 levels. Data within bars indicate median and 25th to 75th percentile. Tertiles of FT4 levels are as follows: first tertile <0.82 ng/dL, second tertile 0.82 to 1.09 ng/dL, third tertile >1.09 ng/dL. *P < .01 for comparison between third tertile and the other 2 tertiles (first and second tertiles).

4.2. *FT4 levels and BMD are correlated with the number of COPs*

Patients in the third tertiles of FT4 levels had the highest number of circulating CD34+/AP+ and CD34+/OCN+ cells (Fig. 1). The FT4 levels were positively correlated with log-CD34+/AP+ cells ($r = 0.32$, $P < .001$) and log-CD34+/OCN+ cells ($r = 0.36$, $P < .001$) (Fig. 2, upper and lower panels, respectively).

The femoral neck BMD was significantly correlated with the log-number of circulating CD34+/AP+ and CD34+/OCN+ cells ($r = –0.20$, $P = .01$ and $r = –0.27$, $P = .001$, respectively) (Table 2).

Other than FT4 levels and BMD, no additional variables appeared to be significantly correlated with the number of COPs. Patients with a positive history of fragility fractures had a higher number of CD34+/AP+ COPs than those without fractures ($P = .02$).

To identify predictors of the number of COPs, we performed a stepwise regression analysis including FT4, BMD, and history of fragility fractures as independent variables. Free thyroxine and BMD were both independently correlated with the log-number of CD34+/OCN+ cells (model $R = 0.39$, $P < .001$), whereas FT4 and history of fragility fractures were correlated with the log-number of CD34+/AP+ cells (model $R = 0.30$, $P = .002$). No evidence of colinearity between independent variables was present in all multivariate models (variance inflation factor [VIF] < 1.1 for all regression analyses).

Forcing in the model age, BMI, 25-OH-vitamin D, bone AP, and CTX did not affect the association between FT4 and COP levels.

4.3. *Additional correlates of BMD*

Free thyroxine levels were correlated with total femur BMD ($r = –0.17$, $P = .036$), but not with femoral neck BMD ($r = –0.14$, $P = .08$) (Fig. 3). Femoral neck BMD was also significantly correlated with BMI ($r = 0.31$, $P < .001$), 25-OH-vitamin D ($\rho = 0.17$, $P = .03$), CTX ($\rho = –0.30$, $P < .001$), and the number of COPs (Table 2).

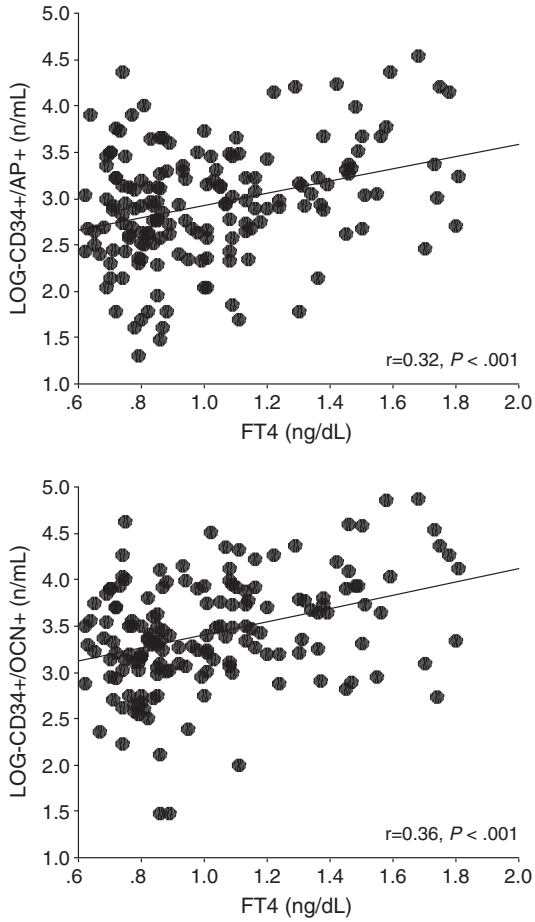

Fig. 2 – Correlation between FT4 levels and either LOG-CD34+/AP+ cells (upper panel) or log-CD34+/OCN+ cells (lower panel).

In the stepwise regression analysis, the model including age, BMI, and CTX levels explained the 22% variability of femoral neck BMD (model $R = 0.47$, $P < .001$); neither 25-OH-vitamin D nor bone AP entered the multivariate model. When the number of COPs was included in the multivariate analysis, femoral neck BMD was inversely predicted by either the log-transformed count of CD34+/AP+ (standardized $\beta = –0.22$, $P = .009$) or CD34+/OCN+ (standardized $\beta = –0.30$, $P < .001$) (model $R = 0.52$ and $R = 0.55$, respectively; $P < .001$ for both models). Free thyroxine levels predicted negatively BMD levels (standard-

Table 2 – Significant correlates of femoral neck and total femur BMD

	Femoral neck BMD	Total femur BMD
BMI [a]	0.31 (<.001)	0.46 (<.001)
25-OH-vitamin D [b]	0.17 (.03)	0.17 (.04)
sCTX [b]	–0.30 (<.001)	–0.31 (<.001)
Log-CD34+/AP+ cells [a]	–0.20 (.01)	–0.18 (.03)
Log-CD34+/OCN+ cells [a]	–0.27 (.001)	–0.24 (.004)

Significance of correlations is indicated in parentheses.
[a] Values are Pearson correlation coefficients.
[b] Values are Spearman correlation coefficients.

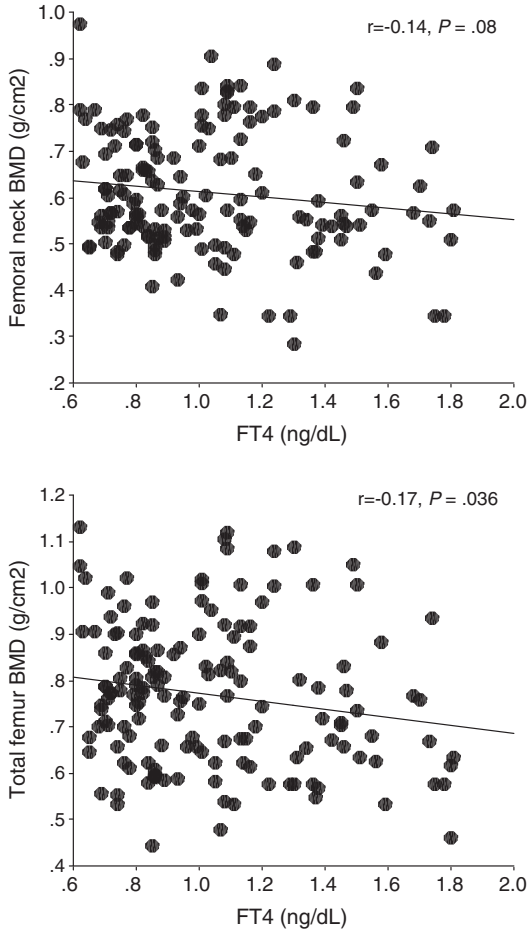

Fig. 3 – Correlation between FT4 levels and either femoral neck (upper panel) or total femur BMD (lower panel).

ized $\beta = -0.21$, $P = .02$) in the multivariate model including age, BMI, 25-OH-vitamin D, bone AP, and CTX; no evidence of collinearity between independent variables was found (VIF < 1.1 for all regression analyses). Comparable results were obtained when replacing femoral neck BMD with total femur BMD (results not shown).

4.4. *Additional correlates of FT4*

Other than 25-OH-vitamin D, COP numbers, and BMD, additional correlates of FT4 levels included age ($r = 0.17$, $P = .04$) and BMI ($r = 0.22$, $P = .006$). The correlation between FT4 levels and age was abolished after adjusting for 25-OH-vitamin D levels ($r = 0.13$, $P = .12$). The correlation between FT4 levels and BMI was independent of the confounding effect of age and BMD ($P < .05$).

4.5. *Effects of triiodothyronine on mineralization by CD34+ cells*

CD34+ cells were exposed to either the osteogenic stimulatory medium alone or to the medium to which increasing concentrations of triiodothyronine (from 0.5 to 10 pmol/L) were added. After 21 days of culture, cells were stained with OsteoImage. Microscopic evaluation indicated a dose-dependent effect of triiodothyronine on mineral deposition (Fig. 4A-G). The exposure of CD34+ cells to triiodothyronine 10 pmol/L was associated with a 26% increase in mineral deposition compared with exposure to osteogenic medium alone.

5. Discussion

Despite agreement that hyperthyroid states may profoundly affect the function of osteoblasts in vitro [8-15] and influence bone formation and resorption with net bone loss in vivo [16,17], the association between physiological variations of thyroid hormone levels, BMD, and circulating osteoprogenitor cells has not been clarified in postmenopausal women. Our findings suggest that FT4 levels, although weakly correlated with bone loss, are also significantly correlated with an increased number of COPs and an increased ability of CD34+ cells to form mineralized nodules in vitro.

To the best of our knowledge, this is the first report of an association between COPs and FT4 levels in postmenopausal women. We have recently found that women with postmenopausal osteoporosis have an increased number of circulating CD34+ COPs, and this suggested low BMD as a possible trigger to stimulate bone marrow to mobilize immature cells with osteogenic potential [6]. In the present study, we confirmed low BMD as a significant covariate of an increased COP number; but we also indicate thyroid hormones as a potential stimulus to increase the number of COPs.

Although the observational design of the study does not allow us to reach conclusions on the mechanism underlying the association between FT4 and COPs, at least 3 lines of evidence support this association. First, osteoblasts express thyroid hormone receptors [24]. Second, thyroid hormone may increase the hematopoietic progenitor number and clonogenic potential [21]. Third, several in vitro studies showed that thyroid hormones increase the differentiation of osteoblastic cell lines [8-13].

Previous demonstrations of a positive effect of thyroid hormones on in vitro mineralization [8-13] are supported by our in vitro results showing an increased ability of circulating CD34+ cells to form mineralized nodules after exposure to physiological doses of triiodothyronine. Hence, not only mature osteoblast cell lines but also immature hematopoietic progenitors expressing the CD34 stem antigen are stimulated by triiodothyronine to produce mineralized nodules in vitro. Our in vitro results might be further supported by the observation that CD34+ hematopoietic progenitors also express thyroid hormone receptors [25].

A negative influence of thyroid hormones on in vitro mineralization has been described [14,15]. Furthermore, high triiodothyronine doses resulted in a negative effect on osteogenic differentiation and less collagen synthesis in rats [14]. Hence, despite the variable effects of different doses of thyroid hormones on osteoblast function in vitro [8-15], it is possible that physiological elevations in FT4 levels may exert a positive influence on the number of COPs by a mechanism that still needs to be completely clarified.

Other than FT4 levels, low BMD also appears to be a significant predictor of the increased count of COPs in the present study. Accordingly, stepwise regression analysis

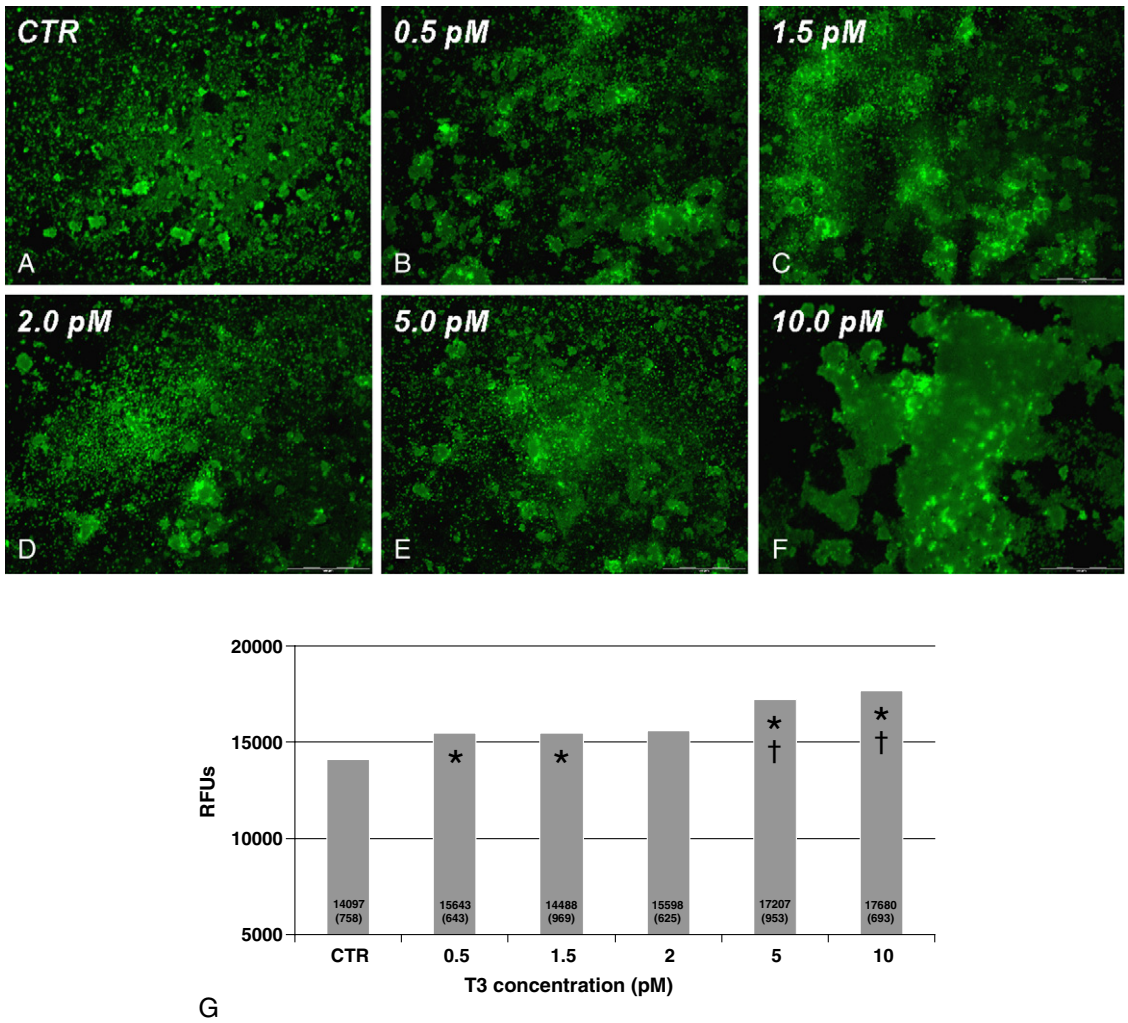

Fig. 4 – Fluorescence microphotographs showing mineral deposition in the absence of triiodothyronine (A) and with increasing concentrations of triiodothyronine from 0.5 to 10 pmol/L (B-F). Arrows indicate fluorescent areas of mineralization. Mean plates fluorescence expressed in RFUs in the absence of triiodothyronine and with increasing concentrations of triiodothyronine (G). In panel G, average results of 5 separate experiments are presented. *P* for linear trend = .003. *P < .05 for comparisons with the control (CTR) group. †P < .05 for comparisons with 0.5-, 1.5-, and 2-pmol/L groups. Values inside the bars are means and SD.

showed that, along with FT4 levels, BMD was negatively correlated with the COP count, independent of FT4 levels and traditional risk factors for osteoporosis. Our study does not provide mechanistic explanations of the latter association and does not indicate in which direction this association should be considered. Therefore, our hypothesis that low BMD in postmenopausal women could be a trigger for COP mobilization into circulation in the attempt to restore an adequate bone mass is speculative. However, the observation that circulating CD34+ cells enhance osteogenesis and form mineralized nodules in vitro [1,2] supports our hypothesis.

Our study also involved assessment of the association between FT4 levels within the reference range and BMD. We found a negative, albeit weak, association between FT4 and total femur BMD that was independent of traditional risk factors for osteoporosis. However, we did not find any association between FT4 and femoral neck BMD. The results of the present study suggest that high-normal FT4 levels may have a very weak or even no correlation with BMD [26-28]. Our findings in euthyroid patients support previous observations of a negative influence of thyroid hormones on BMD in patients with either overt or subclinical hyperthyroidism [16-20].

Another finding emerging from the present study is that thyroxine levels increase within the normal reference range in postmenopausal women with a higher degree of 25-OH-vitamin D deficiency. The observational design of this study does not allow us to reach firm conclusions on the direction of this association; however, we hypothesized that hypovitaminosis D, a frequent finding among postmenopausal women [29,30], might contribute to the variations of FT4 levels. Accordingly, in multivariate analysis with FT4 as a dependent variable, 25-OH-vitamin D was the only predictor of FT4 levels, independent of a consistent number of confounders. In addition, further support to the inverse association between vitamin D and FT4 levels comes from previous studies showing that vitamin D inhibits iodide uptake in thyroid follicular cells [31,32] and that vitamin D

supplementation in hyperthyroid patients is associated with FT4 level reduction [33].

The potential limitations of our study should be noted. The study was restricted to postmenopausal women of Caucasian origin; thus, results may not be applicable to younger women, men, or other ethnic groups. Moreover, the observational design of the study does not allow us to reach conclusions on the mechanism underlying the observed statistical association between FT4, COP levels, and BMD. Finally, because BMD is the result of a long-term process of bone turnover, a single and transient measurement of FT4 and COP levels might not necessarily reflect a dynamic bone remodeling; hence, studies of the association between variations of the above variables are necessary before definitive conclusions are expressed on this issue.

In conclusion, in postmenopausal women, physiological increases in FT4 levels, possibly sustained by reduced 25-OH-vitamin D levels, are correlated with an increased number of circulating osteoprogenitor cells and an improved ability of the CD34+ cells to form mineralized nodules in vitro. Furthermore, a very mild BMD reduction has been observed in patients with high-normal FT4 levels. A further understanding of the behaviour of COPs and BMD in patients with high-normal FT4 levels might provide additional knowledge on the link beween thyroid hormones and osteoporosis.

Funding

This study was supported by grant no. 2008.021.327 from "Fondazione Cassa di Risparmio di Perugia," Perugia, Italy.

Acknowledgment

The authors thank Mrs Shriyani Worsley and Mr Peter Worsley for their useful comments and suggestions in preparing the manuscript.

Conflict of Interest

None declared.

REFERENCES

[1] Eghbali-Fatourechi GZ, Lamsam J, Fraser DN, et al. Circulating osteoblast-lineage cells in humans. N Engl J Med 2005;352:1959-66.
[2] Eghbali-Fatourechi GZ, Mödder UI, Charatcharoenwitthaya N, et al. Characterization of circulating osteoblast lineage cells in humans. Bone 2007;40:1370-7.
[3] Kumagai K, Vasanji A, Drazba JA, et al. Circulating cells with osteogenic potential are physiologically mobilized into the fracture healing site in the parabiotic mice model. J Orthop Res 2008;26:165-75.
[4] Pignolo RJ, Shore EM. Circulating osteogenic precursor cells. Crit Rev Eukaryot Gene Expr 2010;20:171-80.
[5] Chen JL, Hunt P, McElvain M, et al. Osteoblast precursor cells are found in CD34+ cells from human bone marrow. Stem Cells 1997;15:368-77.
[6] Pirro M, Leli C, Fabbriciani G, et al. Association between circulating osteoprogenitor cell numbers and bone mineral density in postmenopausal osteoporosis. Osteoporos Int 2010;21:297-306.
[7] Otsuru S, Tamai K, Yamazaki T, et al. Circulating bone marrow–derived osteoblast progenitor cells are recruited to the bone-forming site by the CXCR4/stromal cell-derived factor-1 pathway. Stem Cells 2008;26:223-34.
[8] Bassett JH, Williams GR. The molecular actions of thyroid hormone in bone. Trends Endocrinol Metab 2003;14:356-64.
[9] Gouveia CH, Schultz JJ, Bianco AC, Brent GA. Thyroid hormone stimulation of osteocalcin gene expression in ROS 17/2.8 cells is mediated by transcriptional and post-transcriptional mechanisms. J Endocrinol 2001;170:667-75.
[10] Kassem M, Mosekilde L, Eriksen EF. Effects of triiodothyronine on DNA synthesis and differentiation markers of normal human osteoblast-like cells in vitro. Biochem Mol Biol Int 1993;30:779-88.
[11] Milne M, Kang MI, Quail JM, Baran DT. Thyroid hormone excess increases insulin-like growth factor I transcripts in bone marrow cell cultures: divergent effects on vertebral and femoral cell cultures. Endocrinology 1998;139:2527-34.
[12] Pereira RC, Jorgetti V, Canalis E. Triiodothyronine induces collagenase-3 and gelatinase B expression in murine osteoblasts. Am J Physiol 1999;277:E496-504.
[13] Varga F, Rumpler M, Luegmayr E, et al. Triiodothyronine, a regulator of osteoblastic differentiation: depression of histone H4, attenuation of c-fos/c-jun, and induction of osteocalcin expression. Calcif Tissue Int 1997;61:404-11.
[14] Boeloni JN, Ocarino NM, Melo AB, et al. Dose-dependent effects of triiodothyronine on the osteogenic differentiation of rat bone marrow mesenchymal stem cells. Horm Res 2009;72:88-97.
[15] Britto JM, Fenton AJ, Holloway WR, Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology 1994;134:169-76.
[16] Karga H, Papapetrou PD, Korakovouni A, et al. Bone mineral density in hyperthyroidism. Clin Endocrinol (Oxf) 2004;61:466-72.
[17] Tauchmanovà L, Nuzzo V, Del Puente A, et al. Reduced bone mass detected by bone quantitative ultrasonometry and DEXA in pre- and postmenopausal women with endogenous subclinical hyperthyroidism. Maturitas 2004;48:299-306.
[18] Kim DJ, Khang YH, Koh JM, et al. Low normal TSH levels are associated with low bone mineral density in healthy postmenopausal women. Clin Endocrinol (Oxf) 2006;64:86-90.
[19] van de Ven AC, Erdtsieck RJ. Changes of bone mineral density, quantitative ultrasound parameters and markers of bone turnover during treatment of hyperthyroidism. Neth J Med 2008;66:428-32.
[20] Faber J, Jensen IW, Petersen L, et al. Normalization of serum thyrotrophin by means of radioiodine treatment in subclinical hyperthyroidism: effect on bone loss in postmenopausal women. Clin Endocrinol (Oxf) 1998;48:285-90.
[21] Kawa MP, Grymula K, Paczkowska E, et al. Clinical relevance of thyroid dysfunction in human haematopoiesis: biochemical and molecular studies. Eur J Endocrinol 2010;162:295-305.
[22] Wang YH, Liu Y, Maye P, et al. Examination of mineralized nodule formation in living osteoblastic cultures using fluorescent dyes. Biotechnol Prog 2006;22:1697-701.

[23] Römer P, Behr M, Proff P, et al. Effect of strontium on human Runx2+/– osteoblasts from a patient with cleidocranial dysplasia. Eur J Pharmacol 2011;654:195-9.
[24] Rizzoli R, Poser J, Bürgi U. Nuclear thyroid hormone receptors in cultured bone cells. Metabolism 1986;35:71-4.
[25] Grymuła K, Paczkowska E, Dziedziejko VB, et al. The influence of 3,3′,5-triiodo-L-thyronine on human haematopoiesis. Cell Prolif 2007;40:302-15.
[26] Lin JD, Pei D, Hsia TL, et al. The relationship between thyroid function and bone mineral density in euthyroid healthy subjects in Taiwan. Endocr Res 2011;36:1-8.
[27] Murphy E, Glüer CC, Reid DM, et al. Thyroid function within the upper normal range is associated with reduced bone mineral density and an increased risk of nonvertebral fractures in healthy euthyroid postmenopausal women. J Clin Endocrinol Metab 2010;95:3173-81.
[28] Morris MS. The association between serum thyroid-stimulating hormone in its reference range and bone status in postmenopausal American women. Bone 2007;40:1128-34.
[29] Bruyère O, Malaise O, Neuprez A, et al. Prevalence of vitamin D inadequacy in European postmenopausal women. Curr Med Res Opin 2007;23:1939-44.
[30] Binkley N, Ramamurthy R, Krueger D. Low vitamin D status: definition, prevalence, consequences, and correction. Endocrinol Metab Clin North Am 2010;39:287-301.
[31] Lamberg-Allardt C, Valtonen E, Polojärvi M, Stewen P. Characterization of a 1,25-dihydroxy-vitamin D3 receptor in FRTL-5 cells. Evidence for an inhibitory effect of 1,25-dihydroxy-vitamin D3 on thyrotropin-induced iodide uptake. Mol Cell Endocrinol 1991;81:25-31.
[32] Ongphiphadhanakul B, Ebner SA, Fang SL, et al. 1,25-Dihydroxycholecalciferol modulates 3H-thymidine incorporation in FRTL5 cells. J Cell Biochem 1992;49:304-9.
[33] Kawakami-Tani T, Fukawa E, Tanaka H, et al. Effect of 1 alpha-hydroxyvitamin D3 on serum levels of thyroid hormones in hyperthyroid patients with untreated Graves' disease. Metabolism 1997;46:1184-8.