Thyroid hormone stimulates basal and interleukin (IL)-1-induced IL-6 production in human bone marrow stromal cells: a possible mediator of thyroid hormone-induced bone loss

C-H Kim, H-K Kim, Y K Shong, K-U Lee and **G S Kim**

Division of Endocrinology, Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea

(Requests for offprints should be addressed to G S Kim, Division of Endocrinology, Asan Medical Center, Song-Pa, PO Box 145, Seoul 138–600, Korea)
(C-H Kim is now at Department of Internal Medicine, Soonchunhyang University College of Medicine, Seoul, Korea)
(H-K Kim is now at Department of Internal Medicine, Gil Medical Center, Gachon Medical College, Inchon, Korea)

Abstract

It is well known that excessive thyroid hormone in the body is associated with bone loss. However, the mechanism by which thyroid hormone affects bone turnover remains unclear. It has been shown that it stimulates osteoclastic bone resorption indirectly via unknown mediators secreted by osteoblasts. To determine if interleukin-6 (IL-6) or interleukin-11 (IL-11) could be the mediator(s) of thyroid hormone-induced bone loss, we studied the effects of 3,5,3′-tri-iodothyronine (T_3) on basal and interleukin-1 (IL-1)-stimulated IL-6/IL-11 production in primary cultured human bone marrow stromal cells. T_3 at 10^{-12}–10^{-8} M concentration significantly increased basal IL-6 production in a dose-dependent manner. It also had an additive effect on IL-1-stimulated IL-6 production, but failed to elicit a detectable effect on basal or IL-1-stimulated IL-11 production. Treatment with 17β-estradiol (10^{-8} M) did not affect the action of T_3 on IL-6/IL-11 production. These results suggest that thyroid hormone may stimulate bone resorption by increasing basal and IL-1-induced IL-6 production from osteoblast-lineage cells, and these effects are independent of estrogen status.

Journal of Endocrinology (1999) **160,** 97–102

Introduction

It has been established that excessive thyroid hormone in the body is associated with bone loss. Bone mass is reduced in patients with hyperthyroidism or receiving long-term thyroid hormone suppression therapy (Toh *et al.* 1985, Diamond *et al.* 1991, Allain & McGregor 1992), and individuals with a history of thyrotoxicosis have an increased risk of fracture (Cummings *et al.* 1995). Studies on biochemical markers (Lee *et al.* 1990, Harvey *et al.* 1991) and bone histomorphometry (Bordier *et al.* 1967, Mundy *et al.* 1976, Mosekilde & Melsen 1978) showed increased bone turnover in thyrotoxic patients, suggesting that predominantly osteoclastic activity is responsible for the bone loss in thyrotoxicosis. However, the exact mechanism by which thyroid hormone stimulates bone resorption remains unclear.

It has been shown that thyroid hormone directly stimulates bone resorption in organ culture of neonatal mouse calvaria (Klaushofer *et al.* 1989) and fetal rat limb bones (Mundy *et al.* 1976, Hoffmann *et al.* 1986). Thyroid hormone receptors have been demonstrated in osteoblastic cell lines such as ROS l7/2·8 (Rizzoli *et al.* 1986), UMR-l06 (LeBron *et al.* 1989) and MC3T3-El (Kasono *et al.* 1988), and direct effects of tri-iodothyronine (T_3) on proliferation and differentiation of human osteoblast-like cells have also been documented (Kassem *et al.* 1993). In contrast, there has been a paucity of data on the direct functional effects of thyroid hormone on osteoclasts, despite a report that human osteoclastoma cells express T_3 receptors (Allain *et al.* 1996). Previous studies suggested that the action of T_3 on bone is mediated by osteoblasts. Allain *et al.* (1992) and Britto *et al.* (1994) showed that T_3 stimulated osteoclastic bone resorption in the presence of osteoblasts, but not in their absence. Furthermore, stimulation of resorption also occurred if osteoblasts were pretreated with T_3 and then osteoblast–osteoclast coculture was carried out in the absence of T_3. These findings imply that thyroid hormone indirectly stimulates osteoclasts via a mediator produced by osteoblasts. However, the mediator responsible for the activation of osteoclasts by thyroid hormone has yet to be elucidated.

Osteoblasts and bone marrow stromal cells produce bone-active cytokines such as interleukin-6 (IL-6) and interleukin-11 (IL-11) which are critical for osteoclast formation and bone resorption (Manolagas 1995, Girasole *et al.* 1992, 1994). These cytokines have been suggested to mediate the effects of many stimulators of bone resorption such as parathyroid hormone, interleukin-1, and tumor necrosis factor-α. On the basis of these studies, it can be

  Printed in Great Britain
0022–0795/99/0160–0097 $10.00/0

postulated that thyroid hormone stimulates IL-6 and/or IL-11 production by osteoblastic cells, resulting in increased bone resorption. To test this hypothesis, we examined the effects of thyroid hormone on basal and IL-1-stimulated IL-6 and IL-11 production in primary cultured human bone marrow stromal cells (hBMSCs). In addition, we investigated the possible influence of estrogen on thyroid hormone action.

Materials and Methods

Materials

3,5,3′-Tri-iodothyronine (T_3), 3,3′,5′-tri-iodothyronine (reverse T_3, rT_3), recombinant human IL-1α and α-modified minimum essential medium (α-MEM) were purchased from Sigma Chemical Co. (St Louis, MO, USA). IL-6 ELISA kits were obtained from Genzyme (Cambridge, MA, USA), and IL-11 ELISA kits from R&D Systems (Minneapolis, MN, USA). The detection limits of IL-6 and IL-11 assays were 18 and 8 pg/ml, and coefficients of variation were 9·5 and 8·9% respectively.

hBMSC culture

hBMSCs were isolated from ribs discarded at the time of open thoracotomy as described previously (Kim *et al.* 1996, 1997*b*). None of the patients from whom the ribs were obtained had metabolic bone disease, thyroid disease, or were taking thyroid hormone. Briefly, the ribs were excised aseptically, cleaned of soft tissues, and opened longitudinally. The exposed bone marrow was flushed out using several washes of serum-free α-MEM. The medium with flushed bone marrow was centrifuged at 1400 r.p.m. for 10 min. Cell pellets were resuspended in culture medium, and enriched bone marrow stromal cells were obtained by Ficoll/Hypaque (specific gravity 1·077) gradient centrifugation. The cells were seeded into a 75 cm^2 plastic culture flask at a density of 4×10^5 cells/cm^2 and cultured in α-MEM containing 10% fetal bovine serum, penicillin and streptomycin (100 U/ml and 100 μg/ml respectively). The medium was changed twice weekly from the second week, and when the cells were grown to 80% confluence, they were then subcultured using 0·01% trypsin and 0·05% EDTA. The second-passage cells were used for the experiments.

Effect of T_3 on basal IL-6 and IL-11 production by hBMSCs

T_3 and rT_3 were dissolved in ethanol and the initial 10^{-5} M stock solution was serially diluted to concentrations of between 10^{-8} M and 10^{-12} M. The vehicle was added to control cultures, and the final concentration of ethanol in the culture medium did not exceed 0·1%. The cells (3×10^4/well) were subcultured in a 48-well plate containing α-MEM and 5% charcoal-stripped serum for 2 days. Subsequently, the medium was replaced with fresh medium containing various doses of T_3 (10^{-12}–10^{-8} M) or rT_3 (10^{-8} M), and then cultured for an additional 72 h. After 72 h, the conditioned medium was collected, centrifuged free of cell debris, and stored at −20 °C until IL-6 and IL-11 assay. The concentration of IL-6 and IL-11 in the medium was measured by ELISA using commercial kits. The number of cells in each well was determined by quadruplicate hemocytometer counts of trypsin–EDTA-released cells at the end of the culture. Data are expressed as the amount of IL-6 or IL-11 produced per 10^5 cells.

Effect of T_3 on IL-1-stimulated IL-6 and IL-11 production by hBMSCs

Cells were seeded into a 48-well plate, cultured as described above, and treated with various doses of IL-l (10, 100, 1000 U/ml) with or without the addition of T_3 (10^{-8} M). Then the amounts of IL-6 and IL-11 produced in the subsequent 72 h of culture were measured in the conditioned medium.

Influence of 17β-estradiol on the effect of thyroid hormone

To examine the possible influence of estrogen on the effect of thyroid hormone on basal and IL-1-stimulated IL-6/IL-11 production, the cells were treated with 17β-estradiol (10^{-8} M) for 24 h before the addition of T_3 and/or IL-1. The results were compared with those from groups not treated with estrogen in the same set of experiments.

Statistics

All experiments were repeated at least three times using different hBMSC preparations, and representative data are shown in the figures. The significance of the differences between treatment groups was assessed using the Mann–Whitney U-test or ANOVA and post-hoc analysis with Duncan's multiple range test as appropriate. Dose–response relationships were examined by Spearman's rank correlation analysis.

Results

Effect of T_3 on basal IL-6 and IL-11 production by hBMSCs

Treatment with T_3, over the concentration range 10^{-12}–10^{-8} M, significantly increased hBMSC IL-6 production in a dose-dependent manner (Fig. 1A). rT_3, an inactive analog of T_3, did not elicit a response at a dose of 10^{-8} M (data not shown).

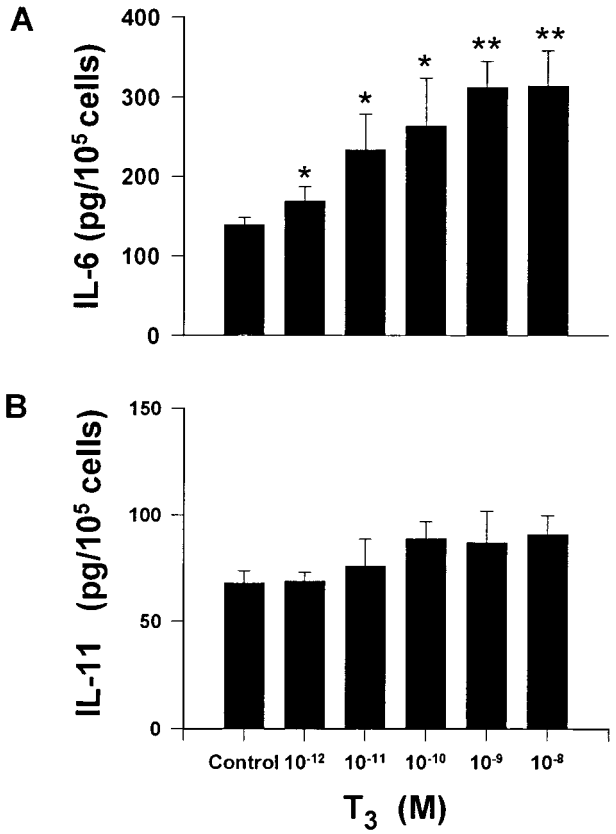

Figure 1 Effects of T_3 on basal IL-6 (A) and IL-11 (B) production in hBMSCs. Cells were treated with various concentrations of T_3 for 72 h, and the amount of IL-6/IL-11 produced in the medium was expressed relative to the number of cells in each well. Values are means ± S.D. (n=6). *P<0·05, **P<0·01 vs control.

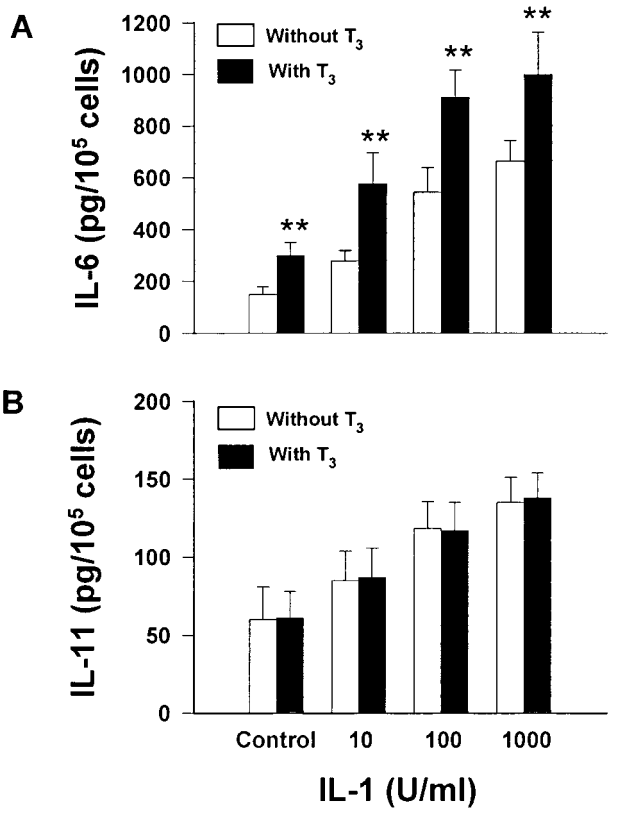

Figure 2 Effects of T_3 on IL-1-induced IL-6 (A) and IL-11 (B) production in hBMSCs. Cells were stimulated with IL-1 (10–1000 U/ml) with or without T_3 (10^{-8} M), and the amounts of IL-6/IL-11 produced during 72 h were measured in the medium. Values are means ± S.D. (n=6). **P<0·01 vs group without T_3.

In contrast, T_3 had no detectable effect on IL-11 production (Fig. 1B).

Effect of T_3 on IL-1-stimulated IL-6 and IL-11 production by hBMSCs

As previously reported (Kim *et al.* 1997*b*), IL-1 (10–1000 U/ml) dose-dependently stimulated both IL-6 and IL-11 production in hBMSCs. T_3 (10^{-8} M) additively increased the IL-1-induced IL-6 production at various concentrations of IL-1 (Fig. 2A). However, IL-1-induced IL-11 production was not affected by T_3 treatment (Fig. 2B).

Influence of 17β-estradiol on the effect of thyroid hormone

Treatment with 17β-estradiol (10^{-8} M) inhibited IL-1-induced IL-6 production, but not T_3-stimulated IL-6 production (Fig. 3A). IL-6 production co-stimulated by T_3 and IL-1 was partially inhibited by 17β-estradiol, but it was still greater than that stimulated by T_3 or IL-1 alone. There was no significant difference in IL-11 production in the presence or absence of 17β-estradiol (Fig. 3B).

Discussion

In the present study, T_3 significantly increased basal and IL-1-stimulated IL-6 production in hBMSCs. These results support the possibility that increased IL-6 production by osteoblast-lineage cells plays an important role in thyroid hormone-induced bone loss. In line with this possibility, Lakatos *et al.* (1997) reported that serum IL-6 concentrations are elevated in patients with hyperthyroidism, and blood mononuclear cells derived from hyperthyroid patients secrete more IL-6 than those taken from healthy subjects. They also showed that bone turnover is increased and radius bone mineral content is reduced in patients with hyperthyroidism. Our results are also consistent with the report of Siddiqi *et al.* (1998) demonstrating that T_3 increased both secretion and mRNA expression of IL-6 and IL-8 by hBMSCs and MG63 osteoblast-like cell lines.

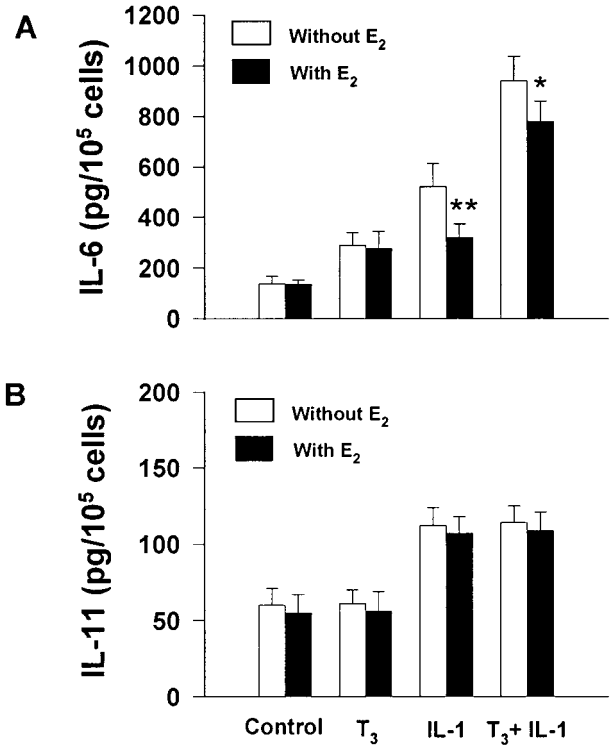

Figure 3 Effects of 17β-estradiol (E_2) on T_3 and/or IL-1-stimulated IL-6 (A) and IL-11 (B) production in hBMSCs. The cells were treated with 17β-estradiol (10^{-8} M) for 24 h before the addition of T_3 (10^{-8} M) and/or IL-1 (1000 U/ml). The amounts of IL-6/IL-11 produced during 72 h were compared with those in groups not treated with estrogen. Values are means ± S.D. (n=6). *P<0·05, **P<0·01 vs group without E_2.

Tarjan & Stern (1994, 1995) reported that T_3 potentiated the stimulatory effect of IL-1 on IL-6 production in neonatal mouse calvarial osteoblasts and fetal rat limb bone cultures, which is in agreement with our results with hBMSCs. However, our results show that T_3 alone significantly increased basal IL-6 production. Tarjan & Stern (1995) failed to observe the stimulatory effect in fetal rat limb bone culture of T_3 on basal IL-6 production over a 10^{-11}–10^{-6} M concentration range. They observed that T_3 alone increased ^{45}Ca release significantly only at a fairly high concentration (10^{-6} M), but in the presence of IL-1, 10^{-8} M T_3 significantly potentiated the IL-1 effect on ^{45}Ca release. Treatment with IL-1 receptor antagonist blocked the potentiating effects of T_3. They therefore speculated that the enhancement of IL-1-induced IL-6 production might be a biologically relevant mechanism of thyroid hormone-induced bone loss. On the other hand, our data showed that T_3, in the absence of IL-1, also directly stimulated IL-6 production. The reason for this discrepancy is not clear, but may be related to the difference in species of cell origin or stage of osteoblast phenotype expression (Williams *et al.* 1994). It has also been suggested that the presence/absence or relative levels of various forms of thyroid hormone receptor (α, β_1, β_2) in osteoblastic cells may explain the variability of the response to thyroid hormone (Suwanwalaikorn *et al.* 1997). Alternatively, we cannot exclude the possibility that endogenous IL-1 production is higher in human than rat bone cell cultures. However, it was reported that human bone marrow stromal cells did not constitutively produce significant amounts of IL-1β, although low levels of expression of IL-1β mRNA were detected (Aman *et al.* 1994).

Our results show that the effect of thyroid hormone on IL-6 production was not affected by estrogen. Consistent with this, Lakatos *et al.* (1997) observed that there was no difference in pre- and post-menopausal IL-6 levels in hyperthyroid patients. Taken together, the results suggest that thyroid hormone and estrogen act via different mechanisms in regulating IL-6 production. A recent meta-analysis report showed that suppressive doses of thyroid hormone therapy had a detrimental effect on all skeletal sites in postmenopausal women, whereas it had little effect in premenopausal women (Uzzan *et al.* 1996). Our experimental data and those of others (Tarjan & Stern 1994, 1995) showing the synergistic effect of thyroid hormone and IL-1, which is increased in the estrogen-deficient state, on IL-6 secretion may explain this phenomenon. However, further studies are needed to clarify this controversial issue.

IL-11 is a fairly recently discovered cytokine which has a role in osteoclast development (Girasole *et al.* 1994). The effects of thyroid hormone on IL-11 secretion have not yet been studied. We observed in this study that neither basal nor IL-1-induced IL-11 production is affected by thyroid hormone. However, we cannot exclude the possibility that thyroid hormone affects IL-11 production synergistically with other systemic or local cofactors (Manolagas 1995, Kim *et al.* 1997*b*) that exist *in vivo*.

In this study, T_3 had a detectable effect on IL-6 secretion at concentrations much lower than those found in human plasma (about 10^{-9} M). Under the experimental conditions used, free hormone concentrations in the medium may be higher than those found *in vivo*, as culture medium containing 5% serum would contain less T_3-binding protein than *in vivo*. At present, however, the physiological implication of this finding is difficult to clarify even if we measure the free T_3 concentrations in the culture medium, because the local thyroid hormone concentration to which bone cells are exposed *in vivo* may be different from the circulating concentration. Further studies are needed.

It should be pointed out that the hBMSCs used in this study are not mature osteoblasts. However, previous studies have shown that, when cultured to confluence in the presence of serum, these cells possess many of the phenotypic characteristics of differentiated osteoblasts, including deposition of mineralized matrix (Kassem *et al.* 1991, Cheng *et al.* 1994). They produce type I procollagen

and osteocalcin in response to 1,25-dihydroxyvitamin D_3 (Kassem *et al.* 1991) and also increase cAMP in response to parathyroid hormone (Cheng *et al.* 1994). We also confirmed that these cells deposit calcium in the extracellular matrix, and express mRNA characteristic of osteoblastic cells, such as alkaline phosphatase, α1(I)collagen, osteopontin, and decorin (Kim *et al.* 1997*a*). Another possible limitation of using these cells is that they may exhibit heterogeneity between samples because of different proportions of preosteogenic, preadipogenic and prechondrogenic cell precursors. However, this should not be a significant problem because we cultured the cells under the same standardized conditions and we repeated the experiments at least three times using different hBMSC preparations to confirm the consistency of the results.

In conclusion, these results suggest that thyroid hormone may increase bone resorption by stimulating osteoclast formation and activity by increasing basal and IL-1-induced IL-6 production by osteoblastic cells.

Acknowledgements

The authors wish to acknowledge the financial support of the Korea Research Foundation in the program year of 1997. The result of this work was presented as an abstract at the 19th Annual Meeting of the American Society for Bone and Mineral Research, Cincinnati, USA, September 1997.

References

Allain TJ & McGregor AM 1992 Thyroid hormones and bone. *Journal of Endocrinology* **139** 9–18.

Allain TJ, Chambers TJ, Flanagan AM & McGregor AM 1992 Triiodothyronine stimulates rat osteoclastic bone resorption by an indirect effect. *Journal of Endocrinology* **133** 327–331.

Allain TJ, Yen PM, Flanagan AM & McGregor AM 1996 The isoform-specific expression of the tri-iodothyronine receptor in osteoblasts and osteoclasts. *European Journal of Clinical Investigation* **26** 418–425.

Aman MJ, Keller U, Derigs G, Mohamadzadeh M, Huber C & Peschel C 1994 Regulation of cytokine expression by interferon-α in human bone marrow stromal cells: inhibition of hematopoietic growth factors and induction of interleukin-1 receptor antagonist. *Blood* **84** 4142–4150.

Bordier P, Miravet L, Matrajt H, Hioco D & Ryckewaert A 1967 Bone changes in adult patients with abnormal thyroid function. *Proceedings of the Royal Society of Medicine* **60** 1132–1134.

Britto JM, Fenton AJ, Holloway WR & Nicholson GC 1994 Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. *Endocrinology* **134** 169–176.

Cheng SL, Yang JW, Rifas L, Zhang S-F & Avioli LV 1994 Differentiation of human bone marrow osteogenic stromal cells *in vitro*: induction of the osteoblast phenotype by dexamethasone. *Endocrinology* **134** 277–286.

Cummings SR, Nevitt MC, Browner WS, Stone K, Fox KM, Ensrud KE, Cauley J, Black D & Vogt TM 1995 Risk factors for hip fracture in white women. *New England Journal of Medicine* **332** 767–773.

Diamond T, Nery N & Hales I 1991 A therapeutic dilemma: suppressive doses of thyroxine significantly reduce bone mineral measurements in both premenopausal and postmenopausal women with thyroid carcinoma. *Journal of Clinical Endocrinology and Metabolism* **72** 1184–1188.

Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC & Manolagas SC 1992 17β-Estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts *in vitro*: a potential mechanism for the antiosteoporotic effect of estrogens. *Journal of Clinical Investigation* **89** 883–891.

Girasole G, Passeri G, Jilka RL & Manolagas SC 1994 Interleukin-11: a new cytokine critical for osteoclast development. *Journal of Clinical Investigation* **93** 1516–1524.

Harvey RD, McHardy KC, Reid IW, Paterson F, Bewsher PD, Duncan A & Robins SP 1991 Measurement of bone collagen degradation in hyperthyroidism and during thyroxine replacement therapy using pyridinium cross-links as specific urinary markers. *Journal of Clinical Endocrinology and Metabolism* **72** 1189–1194.

Hoffmann O, Klaushofer K, Koller K, Peterlik M, Mavreas T & Stern P 1986 Indomethacin inhibits thrombin-, but not thyroxine-stimulated resorption of fetal rat limb bones. *Prostaglandins* **31** 601–608.

Kasono K, Sato K, Han DC, Fujii Y, Tsushima T & Shizume K 1988 Stimulation of alkaline phosphatase activity by thyroid hormone in mouse osteoblast-like cells (MC3T3-E1): a possible mechanism of hyperalkaline phosphatasia in hyperthyroidism. *Bone and Mineral* **4** 355–363.

Kassem M, Risteli L, Mosekilde L, Melsen F & Eriksen EF 1991 Formation of osteoblast-like cells from human mononuclear bone marrow cultures. *APMIS* **99** 269–274.

Kassem M, Mosekilde L & Eriksen EF 1993 Effects of triiodothyronine on DNA synthesis and differentiation markers of normal human osteoblast-like cells *in vitro*. *Biochemistry and Molecular Biology International* **30** 779–788.

Kim C-H, Cheng S-L & Kim GS 1997*a* Lack of autocrine effects of IL-6 on human bone marrow stromal osteoprogenitor cells. *Endocrine Research* **23** 181–190.

Kim GS, Kim C-H, Park JY, Lee K-U & Park CS 1996 Effects of vitamin B_{12} on cell proliferation and cellular alkaline phosphatase activity in human bone marrow stromal osteoprogenitor cells and UMR106 osteoblastic cells. *Metabolism* **45** 1443–1446.

Kim GS, Kim C-H, Choi CS, Park JY & Lee K-U 1997*b* Involvement of different second messengers in parathyroid hormone- and interleukin-1-induced interleukin-6 and interleukin-11 production in human bone marrow stromal cells. *Journal of Bone and Mineral Research* **12** 896–902.

Klaushofer K, Hoffmann O, Gleispach H, Leis H-J, Czerwenka E, Koller K & Peterlik M 1989 Bone-resorbing activity of thyroid hormones is related to prostaglandin production in cultured neonatal mouse calvaria. *Journal of Bone and Mineral Research* **4** 305–312.

Lakatos P, Foldes J, Horvath C, Kiss L, Tatrai A, Takacs I, Tarjan G & Stern PH 1997 Serum interleukin-6 and bone metabolism in patients with thyroid function disorders. *Journal of Clinical Endocrinology and Metabolism* **82** 78–81.

LeBron BA, Pekary AE, Mirell C, Hahn TJ & Hershman JM 1989 Thyroid hormone 5'-deiodinase activity, nuclear binding, and effects on mitogenesis in UMR-106 osteoblastic osteosarcoma cells. *Journal of Bone and Mineral Research* **4** 173–178.

Lee MS, Kim SY, Lee MC, Cho BY, Lee HK, Koh CS & Min HK 1990 Negative correlation between the change in bone mineral density and serum osteocalcin in patients with hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism* **70** 766–770.

Manolagas SC 1995 Role of cytokines in bone resorption. *Bone* **17** 63S–67S.

Mosekilde L & Melsen F 1978 A tetracycline-based histomorphometric evaluation of bone resorption and bone turnover in hyperthyroidism and hyperparathyroidism. *Acta Medica Scandinavica* **204** 97–102.

Mundy GR, Shapiro JL, Bandelin JG, Canalis EM & Raisz LG 1976 Direct stimulation of bone resorption by thyroid hormones. *Journal of Clinical Investigation* **58** 529–534.

Rizzoli R, Poser J & Burgi U 1986 Nuclear thyroid hormone receptors in cultured bone cells. *Metabolism* **35** 71–74.

Siddiqi A, Burrin JM, Wood DF & Monson JP 1998 Tri-iodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. *Journal of Endocrinology* **157** 453–461.

Suwanwalaikorn S, Auken MV, Kang M-I, Alex S, Braverman LE & Baran DT 1997 Site selectivity of osteoblast gene expression response to thyroid hormone localized by *in situ* hybridization. *American Journal of Physiology* **272** E212–E217.

Tarjan G & Stern PH 1994 Triiodothyronine up-regulates the stimulatory effect of interleukin-1β on interleukin-6 production in mouse osteoblasts and fetal rat limb bones. *Journal of Bone and Mineral Research* **9** (Suppl 1) C266 (Abstract).

Tarjan G & Stern PH 1995 Triiodothyronine potentiates the stimulatory effects of interleukin-1β on bone resorption and medium interleukin-6 content in fetal rat limb bone cultures. *Journal of Bone and Mineral Research* **10** 1321-l326.

Toh SH, Claunch BC & Brown PH 1985 Effect of hyperthyroidism and its treatment on bone mineral content. *Archives of Internal Medicine* **145** 883–886.

Uzzan B, Campos J, Cucherat M, Nony P, Boissel JP & Perret GY 1996 Effects on bone mass of long term treatment with thyroid hormones: a meta-analysis. *Journal of Clinical Endocrinology and Metabolism* **81** 4278–4289.

Williams GR, Bland R & Sheppard MC 1994 Characterization of thyroid hormone (T_3) receptors in three osteosarcoma cell lines of distinct osteoblast phenotype: interactions among T_3, vitamin D_3, and retinoid signaling. *Endocrinology* **135** 2375–2385.

Received 8 June 1998
Accepted 1 September 1998