Osteoporotic Cytokines and Bone Metabolism on Rats with Induced Hyperthyroidism; Changes as a Result of Reversal to Euthyroidism

Gönül Simsek¹, Yesari Karter², Seval Aydin³, and Hafize Uzun³

¹Istanbul University
Cerrahpaşa Faculty of Medicine Department of Physiology

²Istanbul University
Cerrahpaşa Faculty of Medicine Department of Internal Medicine

³Istanbul University
Cerrahpaşa Faculty of Medicine Department of Biochemistry
Istanbul, Turkey

Abstract

Hyperthyroidism is characterized by increased bone turnover and resorptive activity. Raised levels of serum osteoporotic cytokines, such as interleukin (IL) -1β, IL-6 and tumor necrosis factor (TNF)-α have been demonstrated previously in hyperthyroidism. These elevations are controversial and it is difficult to differentiate the contribution of thyroid hormones to the elevation of cytokines from that of the autoimmune inflammation in Graves' disease (GD) and follicular cell damage in thyroiditis. Therefore, we investigated the effect of thyroid hormones on serum IL-1β, IL-6, TNF-α levels and bone metabolism on L-thyroxine induced hyperthyroid rats and changes in cytokine levels and bone metabolism on the same rats after reversal to euthyroidism. Rats were treated with L-thyroxine for 5 weeks (0.4 mg/100 g food). Plasma T₃, T₄, TSH and serum IL-1β, IL-6, TNFα, Calcium (Ca), phosphorous (P), parathyroid hormone (PTH), alkaline phosphatase (ALP), bone alkaline phosphatase (B-ALP) levels were measured and differential leucocyte counts were made initially, at the 5th week of the experiment (hyperthyroid state) and 5 weeks after quitting the administration of L-thyroxine (euthyroid state). Significant rises in serum IL-1β, IL-6 and TNFα were noted in hyperthyroidism (P<0.001). In euthyroid state, IL-1β, IL-6 and TNFα decreased significantly, but IL-β and TNFα were significantly higher than the baseline values (P<0.05) while IL-6 levels turned back to the baseline values. Plasma T₃ and T₄ levels were significantly correlated with serum cytokines in hyperthyroid state while there was no correlation in euthyroid states. Ca and P levels did not differ significantly while PTH levels declined significantly in the hyperthyroid state (P<0.05). After the reversal to the euthyroidism, there was no significant change in Ca, P and PTH levels. ALP and B-ALP increased significantly in hyperthyroidism (P<0.001, P<0.01) and they did not decrease in euthyroid state. The lymphocyte number and ratio in differentials increased significantly in the hyperthyroid state (P<0.001). In euthyroidism they decreased significantly (P<0.001) but it was significantly higher than the baseline value (P<0.05). Our findings showed that the deleterious effect on bone metabolism in hyperthyroidism might be mediated by cytokines and the increased bone turnover in hyperthyroidism failed to decrease despite euthyroidism.

Key Words: hyperthyroidism, interleukin-1β, interleukin 6, tumor necrosis factor-α, bone metabolism, bone turnover
Introduction

Thyroid hormones are necessary for normal skeletal growth. However, their excess may lead to bone resorption. Ultimately hyperthyroidism is accompanied by osteoporosis (24, 34). Although there is ample evidence for increased bone turnover in hyperthyroidism (10), the precise mechanisms for this action of thyroid hormone on bone remain unclear.

Locally produced factors are important in maintaining normal bone metabolism (19). IL-6 in particular, has a major influence on bone turnover and stimulates differentiation and proliferation of osteoclasts (16). IL-1β and TNFα are also implicated in bone resorption, particularly in high turnover states (25). Raised levels of serum osteoprotic cytokines such as IL-1β, IL-6 and TNFα have been reported in hyperthyroidism with different etiologies like Graves’ disease (GD), toxic nodular goiter (TNG) and subacute thyroiditis (4, 7, 17). Conversely there exists studies reporting that IL-1β and TNFα have failed to rise in GD and TNG (28, 29). Similarly, in the postpartum thyroiditis (1) and interferon-γ thyroiditis, IL-6 (20) failed to increase.

Serum IL-6 has a number of sources, including blood mononuclear cells and bone tissue. IL-6 mRNA is also present in thyroid follicles (13). In thyroid hyperfunction like TNG, intrathyroidal production of IL-6 is one source of elevated IL-6 levels in serum (17). In GD although follicular cells are also able to express certain cytokines, intrathyroidal lymphocytes are the main source of IL-6 production (36). Serum IL-6 normalizes on remission of the subacute thyroiditis, indicating that it is related to follicular cell damage (17). The data about the levels of cytokines in hyperthyroidism are controversial and it has remained difficult to differentiate the contribution of thyroid hormones to these elevations from that of the autoimmune inflammatory process in GD, hyperfunction of the gland in TNG and follicular cell damage in thyroiditis. To address this question, we investigated the effects of thyroid hormones on serum IL-1β, IL-6 and TNFα levels and on bone metabolism on L-thyroxine-induced hyperthyroid rats intraindividually. As discrepancy exist in the results of studies to determine whether euthyroidism after medical therapy can completely normalize bone metabolism (18, 23), the changes in cytokines and bone metabolism were also investigated on the same rats that were taken back to euthyroidism.

Materials and Methods

Experimental Design

Fifteen female adult Wistar Albino rats of 250-300 g body weight were supplied from the Laboratory Animal Service of the University of Istanbul. The animals were permitted ad libitum access to standard laboratory chow and tap water. Hyperthyroidism was induced by administration of L-thyroxine (Organon Inc, Istanbul, Turkey) 0.4 mg/100 g food for 5 weeks (15, 30, 31, 35).

Blood samples were taken from tail vein initially, 5 weeks after the administration of L-thyroxine (hyperthyroid state) and 5 weeks after quitting L-thyroxine administration (euthyroid state).

Biochemical Measurements

The plasma T₃, T₄, TSH and serum IL-1β, IL-6 and TNFα calcium (Ca), phosphorous (P), Parathyroid hormone (PTH), alkaline phosphatase (ALP) and bone alkaline phosphatase (B-ALP) were analyzed. Additionally differential leucocyte counts were made. T₃, T₄ and TSH analyses were performed by chemiluminescent enzyme immunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA). IL-1β, IL-6 and TNFα were measured by ELISA (Quantikine, R&D Systems, Minneapolis, MN, USA). The sensitivity for IL-1β, IL-6 and TNFα were 5, 10, 5 pg/mL respectively. Ca, P were measured by autoanalyser (Hitachi 717. Boehringer Mannheim, Germany). PTH was analysed by immuno radiometric assay (Diagnostic Systems Laboratories, Inc., Texac, USA). ALP and B-ALP were determined by a kinetic enzymatic method (Boehringer, Mannheim, Germany). For differential leucocyte counts, blood smears were stained with May-Grunwald and Giemsa Solutions (Merc, Darmstad, Germany). At the same time the total leucocyte number and the total numbers of subgroups in mm³ were determined by hematology analyzer (Beckman Coulter, HMX, USA).

Statistical Analysis

Data were expressed as mean(SD) and analyzed by repeated measures of ANOVA. For adjustment of multiple comparisons Least Significant Difference (LSD) was used. Correlations between plasma T₃/T₄ and serum cytokines in different stages were tested by Pearson-Bravis test and P less than 0.05 was accepted as significant.

Results

After 5 weeks of L-thyroxine administration the measured values of significantly increased T₃, T₄ and significantly decreased TSH served to confirm the establishment of hyperthyroidism in the rats. Five weeks after quitting L-thyroxine administration, T₃, T₄ and TSH values reversed to the baseline values.
in hyperthyroidism the secretion of osteoporotic cytokines from the osteoblast and/or blood mononuclear cells may increase by the effect of increased amount of thyroid hormones.

Hyperthyroidism is a well documented cause of impaired bone turnover characterized by increased osteoblastic and osteoclastic activity, resulting in predominance of bone resorption and in decreased bone mass (5, 24). There is no knowledge on the direct stimulating effects of thyroid hormones on osteoclastic activity. Besides stimulating stromal cell progenitors, these hormones are reported to have important functions in osteoblastic maturation (8). Therefore, the cytokine secretion from stromal cells and osteoblasts may increase by the effect of increased thyroid hormones in hyperthyroidism.

IL-6 is produced by both stromal cells and osteoblastic cells in response to stimulation by the bone-resorbing cytokines IL-1 and TNF (11, 22). IL-6 stimulates bone resorption by enhancing osteoclast proliferation and differentiation (16). In vitro thyroid hormones do not stimulate IL-6 production directly in fetal rat limb bones (33). However in the presence of physiological concentrations of thyroid hormones, the IL-1 stimulated IL-6 response and bone resorption is greatly increased in fetal rat limb bone cultures (33). In our study it was seen that the levels of IL-1β, IL-6 and TNFα increased in the hyperthyroid state. After the reversal of the rats to euthyroidism, we saw that the level of IL-6 arrived at the baseline values while the levels of IL-1β and TNFα decreased significantly, but not to the baseline values (Table 2). This may suggest the stimulation of IL-6 by IL-1 at certain concentrations. As the concentration of IL-1 decreases to the values below these concentrations, it will not stimulate IL-6 any more.

Blood mononuclear cells produce IL-6 under normal basal conditions (11). Lakatos et al. (17)
showed the increasing of basal IL-6 secretion from mononuclear cells in GD and TNG. Horowitz et al. (14) reported that IL-1β and TNFα secreted from peripheral monocytes induced the secretion of IL-6 by stimulating stromal cells and osteoblast. In our study lymphocytosis was observed in hyperthyroid rats while there was no change in monocyte number and ratio. We also showed lymphocytosis in hyperthyroidism in our previous study (31). IL-1β is also secreted from lymphocytes (9). Increased numbers of lymphocytes encountered in hyperthyroidism may be responsible for high IL-1β levels. This may lead to elevated IL-6 levels released from activated osteoblasts. After the reversal of the rats to euthyroidism the number of lymphocyte decreased significantly, but it was noticable that these values were significantly greater than the baseline values.

Barnefalk et al. (3) found out that high plasma Ca concentration increased the secretion of IL-6 from mononuclear blood cells, but in our study hyperthyroid rats showed no significant increase in serum Ca levels while there were significant increases in IL-1β, IL-6 and TNFα levels. We also demonstrated that thyroid hormones did not cause significant changes in the plasma level of Ca in our previous studies (30, 31, 32).

Thyroid hormones given in supraphysiological doses result in a reduction in net bone tissue mass by an accentuation in remodelling, causing increased resorative activity (2). Enhanced bone destruction results in a slight rise in serum Ca, which in turn causes a compensatory reduction in the PTH level and augmentation of renal P retention (27). Our findings of insignificant increases in serum Ca and P levels and a significant decline in PTH are consistent with these data. Reversal to euthyroidism did not cause any change in Ca, P and PTH levels (Table 2).

Table 2. Serum levels of measured parameters in baseline, hyperthyroid and euthyroid states of rats
(means ± SD; n=15)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Hyperthyroid</th>
<th>Euthyroid</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β (pg/mL)</td>
<td>807.08±46.87</td>
<td>967.50±40.92</td>
<td>860.58±81.23</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>1007.50±69.16</td>
<td>1111.91±67.34</td>
<td>1001.66±75.17</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
<tr>
<td>TNFα (pg/mL)</td>
<td>510.00±29.07</td>
<td>559.16±30.80</td>
<td>525.41±31.43</td>
<td>&lt;0.001</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>9.19±0.85</td>
<td>9.46±0.98</td>
<td>9.20±0.90</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>7.45±0.87</td>
<td>7.70±0.66</td>
<td>7.62±0.66</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>PTH (mg/dL)</td>
<td>2.91±0.39</td>
<td>2.73±0.37</td>
<td>2.80±0.37</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>189.75±39.18</td>
<td>219.0±42.16</td>
<td>217.50±42.24</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B-ALP (U/L)</td>
<td>27.50±4.48</td>
<td>37.25±8.41</td>
<td>36.91±8.40</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

P<sup>a</sup>, difference of hyperthyroid state from baseline; P<sup>b</sup>, difference of euthyroid state from hyperthyroid state; P<sup>c</sup>, difference of euthyroid state from baseline; NS, not significant

Fig. 1. The correlations between the levels of plasma T<sub>3</sub>/T<sub>4</sub> and serum cytokines in hyperthyroid state. *P<0.05, **P<0.01
The levels of serum ALP and B-ALP were found to increase significantly in hyperthyroidism in our study. These findings are consistent with the activation of the osteoblasts in cohort with the increasing of osteoclastic activity. However, we did not observe any significant decrease in ALP and B-ALP levels after reversal to euthyroidism (Table 2). Garnero et al. (10) concluded that B-ALP is an important biochemical marker demonstrating the increase in bone turnover. Our findings showed that increased bone turnover in hyperthyroidism failed to decrease despite euthyroidism.

The deleterious effects on bone tissue in hyperthyroidism may be mediated by IL-1β, IL-6 and TNFα. Despite the high bone turnover, the reversal of IL-6 to the baseline values suggests that IL-6, may not a sensitive indicator for high bone turnover. It will be important to investigate for the mechanism responsible for the ongoing high bone turnover despite euthyroidism.

References