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# THE INFLUENCE OF EXPERIMENTAL HYPERTHYROIDISM AND HYPOTHYROIDISM ON MOUSE BONE TISSUE

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## ABSTRACT

The effect of L-thyroxine and sodium perchlorate on bone tissue in a 64-day long experiment was studied on 12 male mice. It was found that L-thyroxine caused a significant increase in the numerical areal density of osteoclasts and in the volume density of bone marrow cavities, while the volume density of mineralised bone was significantly decreased. The application of perchlorate caused a significant decrease in the numerical areal density of osteoclasts, in the volume density of bone marrow cavities and osteoid; the volume density of mineralised bone tissue was significantly increased in this condition.

Key words: bone tissue, hypothyroidism, hyperthyroidism, mouse

## INTRODUCTION

Bone and mineral metabolism are influenced by thyroid hormones. Alterations in thyroid hormones secretion leading to hyperthyroidism or hypothyroidism are associated with changes in growth and maturation of the skeleton. Hyperthyroidism increases remodelling activity in trabecular and cortical bone (Ross, 1987). In hypothyroidism bone dynamics are also altered, the trabecular resorption surface is lower and bone cortical thickness is increased (Coindre et al., 1986). These data are valid for human patients.

The aim of this experimental study was to investigate the quantitative morphological changes occurring in mouse backbone microscopic structure after the influence of both increased and reduced blood concentration of thyroid hormones.

## MATERIALS AND METHODS

This experiment was carried out on 12 male mice, Balbc/c strain, 6-8 weeks old. The mice were randomly divided into three groups of 4 animals each. Group 1 was given 1.2% sodium perchlorate ($NaClO_4$) in drinking water in order to reduce the thyroid hormone blood concentrations, group 2 was given 0.02%o L-thyroxine in drinking water in order to increase the thyroxine blood concentration and group 3 was the control group drinking tap water. The animals were fed with pellets for laboratory animals. After being treated for 64 days the animals were sacrificed. One lumbar vertebra of each animal was fixed in 10% formaldehyde, embedded in methyl methacrylate and cut with a Jung K microtome (Reichert) for hard tissue. The fixation causes shrinkage of soft tissues but this is not valid for mineralised bone tissue. 4 $\mu$m thick sections were stained with Goldner's method, which enables differentiation of calcified from uncalcified tissue.

Blood was obtained from the femoralis vein of each animal sacrified under deep ether narcosis. The blood from animals in each group was pooled. Serum concentrations of calcium ions and calcitonin were measured. Variability estimation was based on three performed parallel determinations of Ca++. Calcium was determined photometrically with cresolphthalein complex. The concentration of calcitonin was measured by radioimmunoassay only once. In a previous experiment performed under the same conditions the concentration of hormones $T_4$ and $T_3$ were measured (Logonder-Mlinšek and Kališnik 1991). The lumbar vertebra were stereologically analysed with Weibel's M-42 multipurpose test system (Weibel, 1979, 1980). The volume densities of cartilage, calcified bone tissue, osteoid and bone marrow cavities were estimated under an objective magnification of x63. The numerical areal densities of osteoclasts were estimated astereologically at the same magnification (Kališnik, 1992). The data were statistically evaluated using Student's t-test.

## RESULTS

The results of calcium and calcitonin concentrations are given in figure 1. The concentration of calcium was significantly higher in the thyroxine group in comparison with the perchlorate and control ones, but there were no differences between the control and perchlorate group. The calcitonin concentration was 3.5 times higher in the thyroxine group than in the perchlorate group, and 2.5 times higher than in the control group.

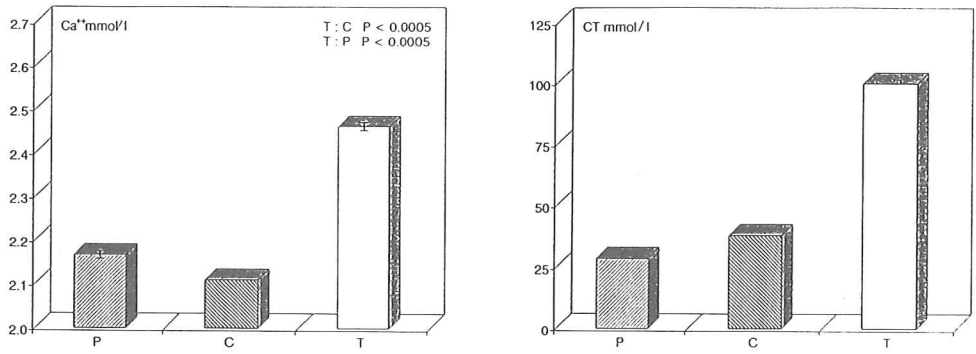


Fig. 1. The concentrations of calcium ions ($Ca^{++}$)(x ± 1SEM) and calcitonin (CT) in the blood serum after 64 days of the experiment in the perchlorate (P), control (C) and thyroxine (T) group.

Quantitative results obtained by stereological analysis show that in the perchlorate group the volume density of mineralised bone tissue was significantly higher than in the control and thyroxine groups ($P<0.001$) (Fig. 2), while the volume density of the bone marrow cavities was significantly higher in the thyroxine group than in the other two groups ($P<0.001$) (Fig. 2). The volume density of osteoid was significantly lower in the perchlorate group in comparison with the control and thyroxine groups ($P<0.001$) (Fig. 3). No differences in the volume density of osteoid were found between the control and thyroxine groups. The greatest numerical areal density of osteoclasts was observed in the thyroxine group and the smallest in the perchlorate group (Fig. 3). There were no differences in size of osteoclasts between all three experimental groups.

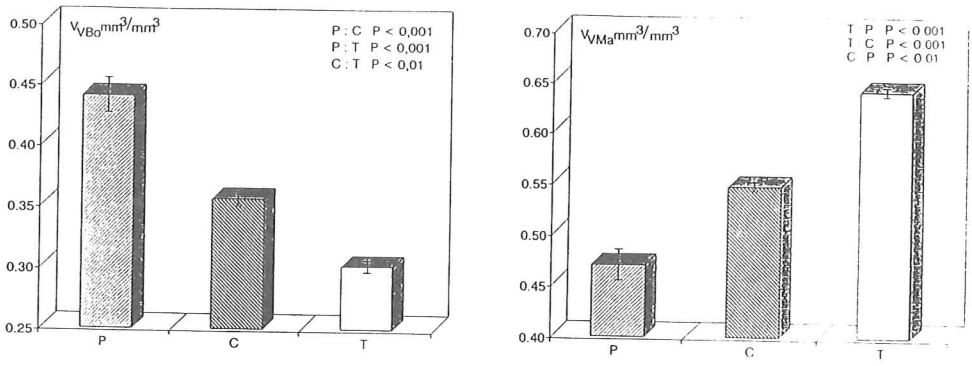


Fig. 2. The volume densities of mineralised bone tissue ($V_{VBo}$) and the bone marrow cavities ($V_{VMa}$) (x ± 1SEM) in three experimental groups.

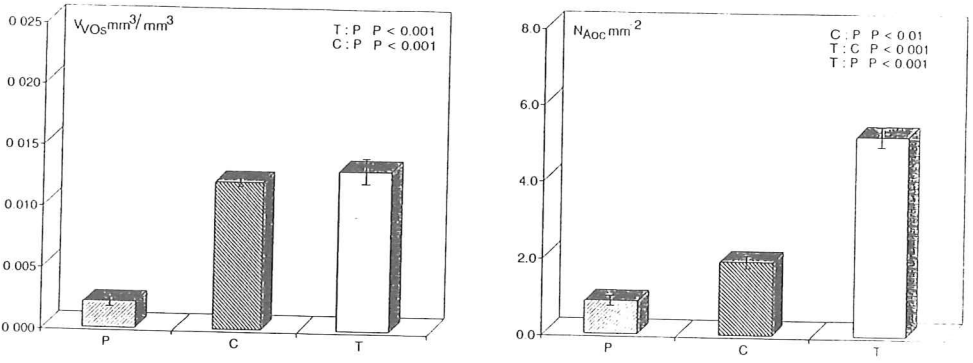


Fig. 3. The volume densities of osteoid ($V_{VOs}$) and numerical areal densities of osteoclasts ($N_{Aoc}$) (x ± 1SEM) in three experimental groups.

## DISCUSSION

The present study demonstrates quantitative morphological changes in lumbar bone tissue in the hyperthyroid (thyroxine group) and hypothyroid (perchlorate group) condition.

We proved that the excess of L-thyroxine stimulated osteoclastic bone resorption shown in the significantly increased numerical areal density of osteoclasts in comparison with the control and perchlorate groups. The consequence of the higher activity of osteoclasts was a significantly higher concentration of blood calcium in the thyroxine group. The significantly higher concentration of calcitonin in this group was the consequential response

to the raised level of blood calcium. The literature, as well as our previous research data (Kališnik et al, 1990) showed that the raising of calcium was a specific stimulus for the parafollicular cells causing hyperplasia of these cells which in turn triggered a higher calcit onin level in the serum. Though Chambers and Moore (1983) found out that calcitonin inhibited osteoclastic bone resorption, we established the increased numerical areal density of osteoclasts and at the same time the high concentration of calcitonin in the thyroxine group.

Furthermore we found out the significantly lower volume density of the mineralised bone tissue in the thyroxine group in comparison with the controls and perchlorate ones.

Most literature data are in agreement with our morphological findings. Histomorphometrical study demonstrated that exogenous thyroid hormones (Coindre et al., 1986; Fallon et al.,1983) led to marked osteoclastosis of both trabecular and cortical bone, already within the first month of treatment. The osteoclastosis led to a decrease of bone mass and bone mineral content, respectively. Paul et al. (1988) found that the bone density of the lumbar spine was unaffected by long term L-thyroxine therapy, while the bone density of the femoral neck was decreased. Seeman et al. (1982) examined both spine and forearm bone density in patients with endogenous hyperthyroidism and found that the spine was more affected. Krolner et al. (1983) reported a decrease in lumbar spine bone density in treatment with L-thyroxine too. Notterstad (1987) found that hyperthyroidism caused bone loss, showing a greater decrease in trabecular than in cortical bone.

Few reports about the bone changes in hypothyroidism were found in the literature. In our perchlorate group the lumbar spine showed the opposite picture to that found in the thyroxine group. The numerical areal density of osteoclasts and the volume density of osteoid were significantly lower while the volume density of mineral bone tissue was significantly increased in comparison with the controls. Coindre et al. (1986) found a lower trabecular resorption surface and increased cortical thickness in hypothyroid patients but a decrease of osteoid volume. But during the first month of hormone replacement hypothyroid patients had osteoclastic hyperresorption in trabecular and cortical bone. Ribot et al. (1990) suggested that in the cases of primary hypothyroidism appropriate thyroid replacement therapy could lead to a significant reduction in vertebral and femoral bone mineral density during the first year of treatment.

Mosekilde and Melsen (1978) established that the osteoclastic resorption in cortical bone as well as the osteoid volume decreased in hypothyroid patients and increased in hyperthyroid patients.

In conclusion, our experiment on mice has confirmed that hyperthyroidism is associated with accelerated loss of bone mass, demonstrated by decreased bone mineral content, increased number of osteoclasts and unexpected high level of calcitonin, while hypothyroidism has the opposite effect.

## ACKNOWLEDGMENTS

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