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# BONE SITE-DEPENDENT EFFECTS ON FEMUR AND VERTEBRAE OF FEMALE RATS WITH THYROID DYSFUNCTIONS

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## ABSTRACT

**Objective:** Evaluating bone site-dependent differences in the effect of thyroid dysfunctions on the femur and lumbar vertebrae of female rats. **Methods:** Thirty-three 2-month-old female wistar rats were distributed in three groups: euthyroid (control), hypothyroid and hyperthyroid. Ninety days after treatment for hypothyroidism and hyperthyroidism induction, the female rats were euthanized; the blood was collected for free T4 dosage and the femurs and segment 1-3 of the lumbar vertebrae were decalcified and processed for analysis of the trabecular bone percentage. **Results:**

The hyperthyroid group showed significantly higher trabecular bone percentage in the femoral metaphysis, in comparison with the control group. But the hyperthyroidism group did not increase the trabecular bone percentage in the lumbar vertebrae. The hypothyroidism group significantly reduced the trabecular bone percentage in the lumbar vertebrae, but did not alter the trabecular bone percentage in the femur. **Conclusion:** The effect of hypothyroidism and hyperthyroidism on bone histomorphometry is different in each condition and bone site-dependent.

**Keywords:** Hypothyroidism. Hyperthyroidism. Femur. Spine. Rats.

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## INTRODUCTION

The thyroid hormones, represented by thyroxine (T4) and by triiodothyronine (T3), are responsible for growth, differentiation and metabolism in the postnatal life of many organs and tissues, including the bone tissue.<sup>1</sup> In the adult bone tissue, they stimulate both bone formation<sup>2</sup> and resorption<sup>3</sup> as they regulate both the activity of the osteoblasts and of the osteoclasts.<sup>4</sup>

The effect of thyroid dysfunctions on the bone tissue, particularly that of hyperthyroidism, is studied extensively in animal models<sup>2,5-13</sup> and in human beings.<sup>14-19</sup> In experimental hyperthyroidism the response of the bone depends on the dose of thyroxine administered, on the serum profile of the sex steroids and of the course of the disease.<sup>2</sup> Some surveys demonstrate that adult rats submitted to high doses of thyroxine present osteopenia.<sup>5-7</sup> However, without measuring the quantity of bone, there are results that suggest that thyroxine administration can increase the bone mass by stimulating the

osteoblastic activity when administered for a short period, or reduce bone mass when administered for a long period.<sup>2</sup> Moreover, thyroxine administration can improve or exacerbate post-castration osteopenia depending on the administration time<sup>2</sup> and on the dose.<sup>9</sup>

Contrary to hyperthyroidism, in hypothyroidism, studied little in the adult bone, osteopenia occurs as a result of inhibition of bone matrix synthesis and of increase of bone resorption in castrated and non-castrated female rats.<sup>12</sup>

Studies suggest that different sites of the skeleton respond in a differentiated manner to the thyroid hormones<sup>5-7,9,20</sup> However, most surveys have performed a merely descriptive evaluation of the effects of hypothyroidism<sup>12</sup> and of hyperthyroidism<sup>2</sup> on the skeleton or a morphometric evaluation of a single bone site.<sup>10</sup> In addition, surveys on the effect of hypothyroidism on the skeleton focus on this effect in the growth phase and not in the adult individual.<sup>21-24</sup>

All the authors declare that there is no potential conflict of interest referring to this article.

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Among surveys on the effects of thyroid dysfunctions on the bone tissue, hyperthyroidism has received special emphasis for several years.<sup>14-17,19</sup> However, hypothyroidism, considered one of the thyroid dysfunctions most frequently diagnosed in human beings, has its effects on the bone tissue of adult individuals studied in a limited manner. Thus the aim of this study was to evaluate the bone site-dependent differences resulting from the effect of thyroid dysfunctions on the percentage of trabecular bone tissue of the femur and of the lumbar vertebrae of female rats.

## MATERIALS AND METHODS

The study subjects were 33 two-month-old female Wistar rats, accommodated in plastic boxes (five to six female rats/box), receiving commercial feed (22% of crude protein, 1.4% of calcium, 0.6% of phosphorus, besides micronutrients) and water *ad libitum*. The rats were kept on a regime of 12 hours of light and 12 hours of darkness. All the procedures described in this study were approved by the Committee of Ethics in Experiments with Animals of Universidade Federal de Minas Gerais - UFMG (Protocol n° 134/2008).

After a thirty day adaptation period, the female rats were divided into three groups: euthyroid (control, n=11), hypothyroid (n=11) and hyperthyroid (n=11). The animals from the hypothyroid group received propyltiouracil (Sigma, Saint Louis, USA) daily through orogastric tube, in the dose of 1 mg/animal, diluted in 5ml of distilled water according to Ribeiro et al., 2004,<sup>12</sup> throughout the experimental period. The animals from the hyperthyroid group received thyroxine (L-thyroxine, Sigma, Saint Louis, USA) daily through orogastric tube, in the dose of 50 µg/animal, diluted in 5ml of distilled water according to Serakides et al.,<sup>2</sup> throughout the experimental period. The animals from the euthyroid group received 5ml of water, as placebo, with the same dosage schedule.

Ninety days after the start of the treatments, the rats were euthanized by cardiac puncture preceded by intraperitoneal anesthesia with pentobarbital 2.5% (30m/kg). The blood was collected for testing the free T4 level by the chemiluminescence technique following the manufacturer's protocol (Access Immunoassay System, Sanofi Diagnostics Pasteur Inc., Chaska, MN, USA), with intratest CV of 4% and 7% and intertest CV of 7% and 11%, respectively. In the postmortem, the femurs and the lumbar vertebrae (L1-L3) were fixed in 10% neutral-buffered formalin and subsequently dissected. The bones were decalcified in a solution of 10% formic acid and subsequently processed by the routine paraffin embedment technique. Histological sections of 5µm were stained by the hematoxylin-eosin (HE) technique for histomorphometric evaluation.

In the femur, the trabecular bone percentage was determined in the epiphysis and distal metaphysis and in the femoral head 1mm below the epiphyseal plate and the articular cartilage, with objective of 20x and with the assistance of an ocular micrometer containing a graticule of 121 points. The variables were determined in three fields in each region, totaling 363 points for every bone site analyzed. In segment 1-3 of the lumbar vertebrae, the trabecular bone percentage was determined in a 20x objective, in a total of 6 fields/vertebra, initiated 1mm below each epiphyseal plate both proximal and distal, totaling 726 points/vertebra.

The experimental delineation was completely random. The mean and the standard deviation were determined for each variable. The data were submitted to the ANOVA of the Instat statistical program (Graph Pad Software, Version 3.00, 32 Win 95/NT, San Diego, USA) and the mean values were compared by the Student-Newman Keuls (SNK) test. Differences were considered significant if  $p < 0.05$ .

## RESULTS

The free T4 levels confirmed the hypothyroid and hyperthyroid states of the animals, since the plasmatic levels of thyroxine were significantly higher in the group treated with thyroxine and significantly lower in the group treated with propyltiouracil in comparison with the control group. (Table 1)

**Table 1 – Plasmatic concentration of free T4 (ng/dl) in female rats from the euthyroid (control), hypothyroid and hyperthyroid groups.**

Group	Concentration of free T4 (ng/dl)
Euthyroid	1.62 ± 0.36 B
Hypothyroid	0.03 ± 0.04 C
Hyperthyroid	4.13 ± 0.99 A

\*Mean values with different letters in the column differ significantly from one another ( $p < 0.05$ ).

In comparison with the control group, hypothyroidism induced for three months significantly reduced the percentage of trabecular tissue only of the lumbar vertebrae. (Table 2) At this bone site, the trabeculas appeared thin, fragmented and hardly connected to one another with fusiform osteoblastic cover (hardly active). In the femur, hypothyroidism did not significantly alter the trabecular bone percentage in any of the regions studied (metaphysis, epiphysis and head). (Table 2)

**Table 2 – Trabecular bone percentage in the femurs and lumbar vertebrae 1-3 of female rats from the euthyroid (control), hypothyroid and hyperthyroid groups.**

Bone site	Group		
	Euthyroid	Hypothyroid	Hyperthyroid
Head (femur)	49.24 ± 4.49 A	51.54 ± 10.56 A	51.04 ± 7.63 A
Distal metaphysis (femur)	37.37 ± 10.37 B	35.49 ± 6.69 B	45.11 ± 7.58 A
Distal epiphysis (femur)	32.23 ± 6.08 A	29.58 ± 2.83 A	31.59 ± 6.97 A
Lumbar vertebrae 1-3	46.33 ± 2.53 A	39.85 ± 3.58 B	46.67 ± 4.64 A

\*Mean values followed by different letters on the line differ significantly from one another ( $p < 0.05$ ).

In the hyperthyroid group, the trabecular bone percentage of the femurs was significantly higher than that of the control group in the region of the distal metaphysis. There was no significant difference at the other sites studied (epiphysis and femur head and lumbar vertebrae). (Table 2) The hyperthyroid group presented

predominantly thick and confluent metaphyseal trabeculas, mainly covered by cuboid osteoblasts with large nuclei. There were also osteoblastic hyperplasia foci. The osteocytes appeared active at one moment, with large nuclei lodged in widened periosteocytic lacunae, then inactive at the next moment, with small nuclei lodged in narrow and weakly basophilic lacunae.

## DISCUSSION

The serum levels of thyroxine confirmed the hypo- and hyperthyroid states of the animals, validating the results in relation to the effect of these thyroid dysfunctions on the femurs and lumbar vertebrae. This result was already expected since equal doses were also used satisfactorily for the induction both of hypothyroidism<sup>12</sup> and of hyperthyroidism.<sup>2,11,13</sup>

The effect of hypothyroidism on the femur and the vertebrae partly corroborates those of another study<sup>12</sup>, where the same dose of propyltiouracil was used, only administered for 120 days instead. Similar to this study, Ribeiro et al.,<sup>12</sup> even without performing bone morphometry, suggests that osteopenia is not as evident in the femur and that the most strongly affected sites are the tibia, lumbar vertebrae, maxilla, jaw and nasal bones.

Unlike hypothyroidism, hyperthyroidism increased the trabecular bone percentage in the femur. In a previous study, with a dose four times higher than the dose used in this study, histomorphometric analysis served to demonstrate an increase in the trabecular bone percentage of the ilium of rats treated with thyroxine for 90 days.<sup>10</sup> However, other studies evaluating the effect of hyperthyroidism on femur and lumbar vertebrae, demonstrated that osteopenia occurs in the femur and not in the vertebra,<sup>5,6</sup> unlike the case observed in this study. And this can be explained by the administration time, since in these studies thyroxine was only administered for a month. In the study conducted by Serakides et al.,<sup>2</sup> thyroxine was administered for 30, 60 and 90 days, suggesting, even without quantifying the bone mass, that the bone response depends on the course of the disease, with osteopenia only after 60 days of thyroxine administration.

The anabolic effect of thyroxine administration was also observed in castrated<sup>2,9</sup> and lactating female rats.<sup>13</sup> Rats treated with thyroxine, depending on the dose and on the administration period, do not manifest post-castration or post-lactation osteopenia, prov-

ing the anabolic effect of hyperthyroidism on the bone mass. But the effect of hyperthyroidism is dependent on the bone site, on the dose of thyroxine and on the course of the disease.<sup>2</sup> Female rats treated with thyroxine for a prolonged period or with an elevated dose have presented exacerbation of post-castration osteopenia.<sup>2,9</sup> In women, hyperthyroidism is also considered a risk factor for postmenopausal osteoporosis.<sup>16</sup>

In this study the anabolic effect of thyroxine on the bone tissue was more evident than the resorptive effect. In vitro assays have demonstrated that the addition of thyroid hormones in osteoblast cultures can stimulate the synthesis activity of these cells.<sup>25</sup> But the osteoblast is a cell that derives from the osteogenic differentiation of stem cells and the areas of osteoblastic hyperplasia observed in the femur of the hyperthyroid rats suggests an increase of stem cell differentiation. This claim is supported by recent results that prove that the thyroid hormones, increase in vitro, the osteogenic differentiation of stem cells of the bone marrow in osteoblasts.<sup>26</sup>

Several surveys have studied the effect of hypothyroidism and hyperthyroidism in rats<sup>2,10-13</sup> and in humans,<sup>15-17,19</sup> but most of these surveys took into consideration only one bone site<sup>10</sup> or studied more than one bone without performing histomorphometry.<sup>2</sup> Furthermore, as far as the authors are aware, only one study was conducted to verify the effect of hypothyroidism on the adult bone without, however, proving these effects by histomorphometry.<sup>12</sup> Accordingly, this study provides supplementary results in relation to the data currently existing in literature.

## CONCLUSION

Thyroid dysfunctions cause different effects on the bone and are dependent on the bone site analyzed. Hyperthyroidism increases the trabecular percentage in the distal metaphysis of the femur, while hypothyroidism reduces the percentage of this tissue only in the vertebra.

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