Thyroid Hormones May Influence Cortical Bone Healing Around Titanium Implants: A Histometric Study in Rats

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Background: The aim of the present study was to evaluate, by histometric analysis, the influence of the thyroid hormones, triiodothyronine (T₃) and thyroxine (T₄), on bone healing around titanium implants inserted in rat tibiae.

Methods: Forty-two male Wistar rats were randomly assigned to the experimental groups: G1 = healthy animals (control; N = 15); G2 = hypothyroidism (N = 13); and G3 = hyperthyroidism (N = 14). Once alterations were confirmed by total serum levels of T₃ and T₄, one screw-shaped titanium implant was placed in the rat tibiae. Sixty days later, the animals were sacrificed, and undecalcified sections were obtained. Bone-to-implant contact (BIC), bone area within the limits of the implant threads, and bone density in a 500-μm-wide zone lateral to the implant were obtained separately for the cortical (zone A) and cancellous (zone B) bone regions.

Results: Intergroup analysis demonstrated that thyroid hormones may significantly affect cortical bone healing around titanium implants. Hyperthyroidism significantly increased the area of newly formed bone in zone A (P < 0.05), whereas hypothyroidism significantly decreased the area of newly formed bone and bone density around the implant in zone A (P < 0.05) compared to the healthy group. In addition, hyperthyroidism significantly increased BIC extension in zone A compared to hypothyroidism (P < 0.05).

Conclusion: Thyroid hormones may influence the healing process in the cortical bone around titanium implants placed in rats, whereas cancellous bone seems to be less sensitive to changes in T₃ and T₄ serum levels. J Periodontol 2008;79:881-887.

KEY WORDS
Bone and bones; dental implants; thyroxine; titanium; triiodothyronine; wound healing.

Triiodothyronine (T₃) and thyroxine (T₄) are produced by the thyroid gland and may affect metabolic processes throughout the body.¹ T₃ and T₄ are fundamental for normal bone turnover.² In vitro studies showed that T₃ may act on osteoblasts to indirectly stimulate osteoclastic bone resorption³ and may be important in switching the osteoblast phenotype from a proliferating preosteoblast form to a mature osteoblast phenotype capable of bone matrix formation.⁴ Moreover, thyroid hormones were shown to regulate gene expression in osteoblastic cells.⁵,⁶

Drugs, illness, age, thyroid disease, and pituitary disorders may affect the secretion of T₃ and T₄ from the thyroid gland to the blood. Hypothyroidism and hyperthyroidism, defined by a decrease and increase in the serum levels of the thyroid hormones, respectively, have been linked to altered osteoblast and osteoclast activity, leading to an imbalance in bone turnover.¹,² The association between hyperthyroidism and bone metabolism was first described >100 years ago.⁷ However, it was not until the 1970s that it became apparent that thyroid-associated metabolic bone disease had specific histologic features.⁸ In rats, hyperthyroidism histologically demonstrates an increase in mineral apposition and formation rates and a small increase in the number of eroded surfaces, as well

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as a decrease in bone mineral density (BMD), as determined by dual energy x-ray absorptiometry. The consequences of hypothyroidism on histologic features and on BMD are less well documented. In rats in which hypothyroidism has been induced, there is a marked reduction in osteoid and eroded surfaces and further changes in the epiphyseal growth plate.

Titanium endosseous dental implants are being used more often to restore function and esthetics in partially or fully edentulous patients, and the achievement and maintenance of osseointegration are highly dependent on bone quality and quantity. Previous studies showed that systemic conditions may be correlated with impaired bone healing around titanium implants, especially in metabolic bone diseases such as osteoporosis, and diabetes mellitus. Bone is a highly metabolically active tissue in which the processes of osteoblastic bone formation (anabolic activity) and osteoclastic resorption (catabolic activity) are continuous throughout life. Therefore, the capacity of bone tissue to respond to injuries such as fracture or implant placement is associated with several mechanisms and may be affected by different conditions.

Although thyroid dysfunctions may affect bone metabolism via their effect on thyroid hormone levels that influence bone turnover, there is a lack of information regarding the effect of changes in T3 and T4 serum levels on bone healing around titanium implants. Thus, the purpose of the present study was to investigate, by histometric analysis, the effect of thyroid hormones on bone healing around titanium implants placed in the rat tibiae.

MATERIALS AND METHODS

Animals

The study included 42 male Wistar rats, aged 60 days and weighing an average of 25.71 g at study onset. During the experiment, the animals were housed in groups of five in plastic cages. Food and water were given ad libitum to all animals. Prior to the beginning of the experimental procedures, animals were allowed to acclimatize to the laboratory environment for 5 days. This protocol was approved by the University of Campinas Institutional Animal Care and Use Committee.

Experimental Design

The animals were assigned to one of the following experimental groups: G1 = control healthy animals (N = 15); G2 = hypothyroidism (N = 13; 1 g propylthiouracil / L drinking water); and G3 = hyperthyroidism (N = 14; 800 µg sodium l-thyroxine and 180 µg sodium triiodothyronine / L drinking water). The solutions containing drugs to induce changes in thyroid hormone levels were ingested by the animals during the entire experimental period, without interruption (Fig. 1).

Biochemical Serum Analyses

Ninety days after the beginning of the study, blood samples were collected and stored at −20°C to allow for the assessment of total serum levels of T3 and T4 by radioimmunoassay according to the manufacturer’s instructions.

Implant Surgery

After the establishment of T3 and T4 serum level alterations, screw-shaped machined titanium implants were inserted in tibiae according to a previously described method. Briefly, general anesthesia was obtained by intramuscular administration of ketamine (50 mg/kg) and xylazine (15 mg/kg). The skin was cleansed with iodine surgical soap. An incision of 1.0 cm in length was made, and the bone surface of the tibiae was surgically exposed by blunt dissection. Under profuse saline solution irrigation, bicortical implant beds were drilled at a rotary speed not exceeding 1,500 rpm. A screw-shaped commercially available pure titanium implant, 4.0 mm in length and 2.2 mm in diameter, was placed bilaterally until the screw thread was completely introduced into the bone cortex (Fig. 2). Finally, soft tissues were replaced and sutured. Postoperatively, the animals received antibiotics via a single intramuscular injection (1 ml/kg).

Histometric Procedures

Sixty days after implant placement, the animals were sacrificed, and tibiae were removed and fixed in 4% neutral formalin for 48 hours. Undecalcified sections were prepared as described previously. The blocks

![Figure 1. Experimental design.](image-url)
were dehydrated using an ascending series of ethanol (60% to 100%) and embedded in glycolmethacrylate. Subsequently, sections (20 to 30 μm) were obtained and stained using 1% toluidine blue. A masked, calibrated examiner recorded the percentages of bone-to-implant contact (BIC), bone area (BA) within the limits of the threads of the implants, and bone density in a 500-μm-wide zone lateral to the implant surface (BD) for the cortical (zone A) and cancellous (zone B) bone areas.

**Statistical Analyses**

Data from zones A and B were averaged separately. The hypothesis that there were no differences among the groups with respect to histometric data (BIC, BA, and BD) and hormone serum levels (T₃ and T₄) was tested by one-way analysis of variance (ANOVA) (α = 0.05). If a statistical difference was detected, a pairwise multiple comparison procedure was used (Tukey test). In addition, repeated-measures ANOVA (α = 0.05) was used to test the hypothesis that there were no intra- or intergroup differences over time for animal body weight. The Tukey test was used to detect differences.

**RESULTS**

**Clinical Observations**

Data analysis demonstrated that the initial body weight was similar for all experimental groups (P>0.05). At the end of the study, animals in the control (G1) and hyperthyroidism groups (G3) had a significant increase in body weight compared to the beginning of the study (P<0.05), whereas no difference was detected for animals in the hypothyroidism group (G2) over time (P>0.05). An intergroup analysis also demonstrated that, at the end of the experimental period, the body weights of animals in G1 and G3 were significantly higher than those in G2 (P<0.01) (Table 1). Macroscopic analysis during autopsies confirmed the success of thyroid dysfunction induction. Thyroid glands in G1 and G3 were larger and pink colored, whereas in G2 the glands were thin and anemic as a result of reduced activity. The glands in G3 appeared slightly larger than in G1.

**Biochemical Analyses**

The total serum levels of T₃ and T₄ before implant surgery are summarized in Table 2. T₃ and T₄ serum levels were higher in hyperthyroidism (G3) and decreased in hypothyroidism (G2) compared to control (G1) (P<0.01 and P<0.0001 for T₃ and T₄, respectively).

**Histometric Analyses**

BIC. In zone A, although the hormone level change did not affect BIC compared to the control group, significantly less BIC was promoted by hypothyroidism than by hyperthyroidism (P<0.05). In addition, with regard to BIC, no significant difference was observed in zone B (P>0.05) (Table 3; Fig. 3).

BA. Data analysis showed that T₃ and T₄ serum levels significantly affected BA in zone A (P<0.05).
Hormone levels and the area of newly formed bone within the limits of the implant threads had a positive relationship, i.e., hypothyroidism and hyperthyroidism resulted in significantly decreased and increased BA, respectively. Although there was no statistical difference in this parameter in zone B, a similar trend of increasing bone area with increased hormone levels was observed (Table 4; Fig. 3).

BD. In zone A, intergroup analysis revealed that hypothyroidism significantly reduced the proportion of mineralized tissue adjacent to the implant surface ($P<0.05$). In addition, similar to our findings for the other histometric parameters, zone B was not significantly affected by either condition ($P>0.05$) (Table 5; Fig. 3).

### DISCUSSION

Disorders in $T_3$ and $T_4$ secretion from the thyroid gland are among the most common endocrine maladies. Different populations show a prevalence that ranges between 0.9% and 15.9% and 0.25% and 7.3% for hypothyroidism and hyperthyroidism, respectively. $T_3$ and $T_4$ are known to stimulate bone apposition and resorption, and therefore, changes in the serum levels of these hormones may influence bone remodeling.

No information is available in the literature regarding the role of these hormones in bone healing around titanium implants. In the present study, data analysis showed that thyroid hormones influenced cortical bone healing around titanium implants inserted in rat tibiae, whereas cancellous bone may be less sensitive to $T_3$ and $T_4$ serum level changes; it was found that hypothyroidism and hyperthyroidism may influence bone around titanium implants during the healing phase. Hypothyroidism resulted in less newly formed bone within the limits of the implant threads and in a poorer bone quality adjacent to the implant surface, whereas hyperthyroidism resulted in a significant increase in the area of newly formed bone, compared to the control group. When extreme serum levels of hormones were compared, BIC was significantly increased in hyperthyroidism compared to hypothyroidism. In the current study, the success of thyroid gland dysfunction was clinically confirmed by autopsies and biochemical analyses, demonstrating that the system used was efficient, as previously reported. Finally, the hypothyroid animals did not show a gain of body weight as seen in the other two groups. Similar findings were reported in a previous study and, essentially, decreases in energy expenditure and voluntary food intake have been implicated.

The mechanisms by which thyroid hormones modulate bone remodeling are not fully understood. It has been suggested that the effects of these hormones on osteoclastic bone resorption are indirect and mediated by osteoblasts through the hormone receptors (TRs). Thyroid hormones modulate alkaline phosphatase, osteocalcin, collagen, and certain growth factors and cytokines involved in bone remodeling with respect to new bone formation. It was also shown that an imbalance in the levels of $T_3$ and $T_4$ correlated positively with the levels of the factors involved with bone homeostasis. For instance, a decrease in osteoprogenitor cells, growth factors, and cytokines, resulting in a decreased bone apposition, was reported for hypothyroidism, whereas the opposite characterizes hyperthyroidism. In the present study, a similar trend was observed, meaning that hypothyroidism

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**Table 1.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Body Weight (g)</th>
<th>Final Body Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>201.48 ± 12.69 Aa</td>
<td>445.01 ± 46.47 Ab</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>199.97 ± 23.77 Aa</td>
<td>200.27 ± 23.12 Ba</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>180.06 ± 31.72 Aa</td>
<td>443.89 ± 45.11 Ab</td>
</tr>
</tbody>
</table>

Means followed by different letters indicate significant differences ($\alpha = 0.005$) by repeated-measures ANOVA and the Tukey test.

**Table 2.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Triiodothyronine (ng/dl)</th>
<th>Thyroxine (µg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>108.75 ± 16.03 B</td>
<td>2.62 ± 0.65 B</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>85.94 ± 19.34 C</td>
<td>1.27 ± 0.17 C</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>173.24 ± 34.07 A</td>
<td>4.10 ± 0.80 A</td>
</tr>
</tbody>
</table>

Means followed by different letters indicate significant differences ($\alpha = 0.05$) within each column by one-way ANOVA and the Tukey test.

**Table 3.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Zone A</th>
<th>Zone B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.71 ± 7.27 AB</td>
<td>10.11 ± 5.82 A</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>12.28 ± 9.04 B</td>
<td>16.25 ± 12.88 A</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>20.76 ± 9.30 A</td>
<td>14.47 ± 12.14 A</td>
</tr>
</tbody>
</table>

Means followed by different letters indicate significant differences ($\alpha = 0.05$) within each column by one-way ANOVA and the Tukey test.
and hyperthyroidism resulted in impaired and improved bone healing, respectively.

Some skeletal manifestations of hypothyroidism have also been described. When hypothyroidism is induced in young rats, a delayed skeletal maturation and bone growth retardation is observed,2 whereas in adult rats, a marked reduction in osteoid surfaces and mineral apposition rate is observed during histomorphometric evaluation.7 Moreover, this condition is associated with an increased fracture risk, usually associated with osteopenia in humans.27 Hyperthyroidism, a more extensively investigated condition, results in a decreased bone mineral density in rats, characterized by an increased mineral apposition rate and eroded surfaces.7 However, in humans, an osteopenia or osteoporosis pattern is rarely induced by excessive secretion of T3 and T4 because other, more severe symptoms are detected and treated.28

In general, the skeletal manifestations of thyroid hormones vary depending on the skeletal site.2 The impact of these hormones on long bones, such as tibiae and femurs, has been established; however, such an effect is not observed in other, shorter bones, such as the lumbar spine.7 Moreover, it was suggested that hyperthyroidism and hypothyroidism seem to influence cortical and cancellous bone with a different intensity, with significant changes in cortical bone thickness and no significant effect on cancellous bone.29 Therefore, the data obtained in the present study confirmed that cortical bone is more sensitive than cancellous bone following changes in T3 and T4 serum levels. Potential explanations for the heterogeneous response to thyroid hormones found in the present study regarding cortical and cancellous bone may include modulation of the action of these hormones by: 1) qualitative and quantitative differences in TRs; 2) differential expression of vitamin D3 and retinoid receptors that form heteromeric complexes with thyroid hormone receptors in regulating thyroid hormone action in osteoblasts; and 3) postreceptor modifications in thyroid hormone action.29

### Table 4.

<table>
<thead>
<tr>
<th>Group</th>
<th>Zone A</th>
<th>Zone B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>71.92 ± 5.12 B</td>
<td>42.03 ± 12.64 A</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>65.60 ± 8.76 C</td>
<td>36.97 ± 7.77 A</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>78.72 ± 3.88 A</td>
<td>47.55 ± 13.09 A</td>
</tr>
</tbody>
</table>

Means followed by different letters indicate significant differences (α = 0.05) within each column by one-way ANOVA and the Tukey test.

### Table 5.

<table>
<thead>
<tr>
<th>Group</th>
<th>Zone A</th>
<th>Zone B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>95.93 ± 1.90 A</td>
<td>24.83 ± 16.54 A</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>93.74 ± 2.70 B</td>
<td>24.81 ± 23.40 A</td>
</tr>
<tr>
<td>Hyperthyroidism</td>
<td>95.26 ± 1.56 AB</td>
<td>17.33 ± 8.68 A</td>
</tr>
</tbody>
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Means followed by different letters indicate significant differences (α = 0.05) within each column by one-way ANOVA and the Tukey test.

Figure 3.
Photomicrographs illustrating the histologic aspects observed for control (A), hypothyroidism (B), and hyperthyroidism (C) groups (toluidine blue; original magnification ×2.5; bar = 0.125 mm).
Clinically, thyroid gland diseases may show a wide range of severity and various end-organ effects, such as mental disablement, bone weight alterations, abnormal skeletal development, and increased risk for fractures, resulting in special care for these patients, including dental care. To the best of our knowledge, the present study was the first to evaluate the effect of thyroid hormones on bone healing around titanium implants. The findings presented here clearly demonstrate that clinicians should not underestimate these conditions when dealing with patients diagnosed with thyroid gland dysfunctions who are referred for implant placement.

However, further studies, including long-term clinical trials and investigations on the impact of implant surface on implant outcome, are required before definitive conclusions can be drawn. The events observed in this rat model may not faithfully reproduce events in humans, and extrapolating data should be done with caution.

CONCLUSION
Within the limits of the present investigation, it can be concluded that thyroid hormones may influence the healing process in the cortical bone around titanium implants placed in rats, whereas cancellous bone seems to be less sensitive to changes in T3 and T4 serum levels.

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