DANIELA DA SILVA FEITOSA

Cirurgiã-dentista

INFLUÊNCIA DOS HORMÔNIOS TIREOIDIANOS NA DENSIDADE ÓSSEA E NA PERIODONTITE: ESTUDO EM RATOS

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de doutor em Clínica Odontológica, Área de Periodontia.

Orientador: Prof. Dr. Sérgio de Toledo

PIRACICABA

2009

DANIELA DA SILVA FEITOSA

Cirurgiã-dentista

INFLUÊNCIA DOS HORMÔNIOS TIREOIDIANOS NA DENSIDADE ÓSSEA E NA PERIODONTITE: ESTUDO EM RATOS

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do título de doutor em Clínica Odontológica, Área de Periodontia.

Orientador: Prof. Dr. Sérgio de Toledo

PIRACICABA

2009

FICHA CATALOGRÁFICA ELABORADA PELA

BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA

Bibliotecária: Marilene Girello – CRB-8ª. / 6159

F329i

Feitosa, Daniela da Silva.

Influência dos hormônios tireoidianos na densidade óssea e na periodontite: estudo em ratos. / Daniela da Silva Feitosa. -- Piracicaba, SP: [s.n.], 2009.

Orientador: Sérgio de Toledo.

Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Periodontia. 2. Tireóide. I. Toledo, Sérgio de. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

(mg/fop)

Título em Inglês: The influence of thyroid hormones on bone density and periodontitis: a study in rats

Palavras-chave em Inglês (Keywords): 1. Periodontics. 2. Thyroid

Área de Concentração: Periodontia

Titulação: Doutor em Clínica Odontológica

Banca Examinadora: Sérgio de Toledo, Enilson Antonio Sallum, Francisco Humberto Nociti Júnior, Giuseppe Alexandre Romito, Renata Cimões Jovino Silveira

Data da Defesa: 25-08-2009

Programa de Pós-Graduação em Clínica Odontológica

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 25 de Agosto de 2009, considerou a candidata DANIELA DA SILVA FEITOSA aprovada.

Prof. Dr. SÉRGIO DE TOLEDO

Prof. Dr. GIUSEPPE ALEXANDRE ROMITO

Profa. Dra. RENATA CIMÕES JOVINO SILVEIRA

Prof. Dr. FRANCISCO HUMBERTO NOCITI JÚNIOR

Prof. Dr. ENILSON ANTONIO SALLUM

DEDICATÓRIA

À minha mãe, Socorro, pelo amor e dedicação. Pelo grande exemplo de força e de vida que representa para mim.

AGRADECIMENTOS

Ao Excelentíssimo Senhor Prof. Dr. Fernando Ferreira Costa, magnífico reitor da Universidade Estadual de Campinas.

À Faculdade de Odontologia de Piracicaba - Unicamp na pessoa do seu diretor, Prof. Dr. Francisco Haiter Neto e Diretor Associado, Prof. Dr. Marcelo de Castro Meneghim.

Ao Prof. Dr. Jacks Jorge Júnior, coordenador geral dos cursos de pós-graduação da FOP/UNICAMP.

À Profª. Drª. Renata Cunha Matheus Rodrigues Garcia, coordenadora do curso de pós-graduação em Clínica Odontológica da FOP/UNICAMP.

Ao CNPq e à FAPESP, pelo incentivo financeiro na concessão da bolsa de estudo e por viabilizarem os estudos.

Ao Prof. Dr. Sérgio de Toledo, meu orientador, por ter me recebido e depositado em mim tanta confiança! Pelo exemplo de conduta profissional e pessoal, no qual tento me espelhar. Pela grande amizade! Por quem tenho grande admiração, respeito e carinho.

Ao Prof. Dr. Antônio Wilson Sallum, por nos contagiar com seu entusiasmo pela vida acadêmica e pela generosidade em compartilhar toda sua experiência. Pelo constante incentivo e confiança.

Ao Prof. Dr. Enilson Antonio Sallum, pelo exemplo de profissionalismo e ética. Por me incentivar a trabalhar de maneira autônoma e construir uma vida acadêmica consistente.

Ao Prof. Dr. Francisco Humberto Nociti Júnior, Chico, pelo incentivo e participação neste trabalho. Por me estimular a sempre tentar ultrapassar meus limites, por ter acompanhado meus passos desde o início da pós-graduação. Pelo convivência, pelo exemplo de dedicação e amor ao trabalho e à pesquisa sobretudo, pelo grande aprendizado.

Ao Prof. Dr. Márcio Zaffalon Casati, por quem tenho grande admiração, pela atenção e cuidado sempre dispensados e pela confiança em mim.

Aos professores que participaram da banca de qualificação: Profa. Dra. Daiane Cristina Peruzzo, Prof. Dr. Marcelo Rocha Marques e Prof. Dr.Márcio Zaffalon Casati, por aceitarem prontamente o convite para comporem a banca de qualificação desta dissertação e pelas sugestões oferecidas.

À Prof[a]. Dr[a]. Gláucia Ambrosano pela importante ajuda na realização da estatística.

À Eliete e Regina, por estarem sempre dispostas a ajudar, a esclarecer minhas dúvidas e solucionar meus problemas, pela simpatia e carinho.

À Mari, por sua indispensável ajuda no laboratório. Por tornar o meu trabalho mais leve e divertido e fazer com que as horas passassem mais rápido com as nossas conversas.

A Wanderley, Fábio e Flor, funcionários do biotério, pelos cuidados com os animais e com todos os passos para execução deste trabalho.

Aos colegas de turma, Bia, Fabrícia, Fernanda, Liana, Ezymar, Mauro, Renato, Thaisangela e Wagner. Por uma convivência tranqüila, alegre e marcada por grande companheirismo. Com cada um aprendi um pouquinho, com cada experiência, cada personalidade, cada conversa, cada ajuda...

Aos amigos do doutorado Bruno Benatti, Cléverson de Oliveira, Daiane Peruzzo, Érica Ribeiro, Gabriela da Cruz, Marcelo Carvalho, Roberta Tunes, Sandro Souza e Saulo dos Santos pelo exemplo e companheirismo.

À Fer e ao Re, pelas conversas, risadas e opiniões, pela sua amizade.

Ao Marcelão, pela generosidade e ajuda com a parte laboratorial deste trabalho.

A todos aqueles que me incentivaram e participaram da minha vida profissional antes da pós-graduação, Sérgio Martorelli, Antonio Vildes, Cinthia Gusmão, Silvinha, Lourdes Florio, Rodrigo Veras, Sílvia Jamelli. Espero que possa retribuir toda a confiança depositada em mim.

À Renata Cimões, por ter sido a minha maior torcedora, por depositar tanta confiança em mim, embora eu não mereça, por me acompanhar sempre e ser tão presente, desde a época em que éramos dupla de especialização.

Às incríveis amigas que conquistei durante a vida, minha segunda família, Renata, Luciana, Anna, Andréia, Shynaide e Carmen. Por dividir comigo todas as conquistas e todos os problemas. Tenho muita sorte por ter encontrado vocês no meu caminho.

Ao Mauro, por estar sempre ao meu lado, me incentivando e me ajudando. Por se dedicar a mim com tanto carinho. Obrigada por tornar meus dias melhores e muito mais felizes.

À minha família... quanto maior o amor, mais difícil é expressá-lo em palavras. Eu sou um resultado, talvez não à altura, mas um resultado de vocês.

Obrigada pela construção da minha personalidade e por me dar suporte durante toda a vida.

A todos que contribuíram com este trabalho e com a minha trajetória antes dele.

Meus sinceros agradecimentos...

EPÍGRAFE

"É melhor tentar e falhar,

que preocupar-se e ver a vida passar;

é melhor tentar, ainda que em vão,

que sentar-se fazendo nada até o final.

Eu prefiro na chuva caminhar,

que em dias tristes em casa me esconder.

Prefiro ser feliz, embora louco,

que em conformidade viver ..."

Martin Luther King

Os hormônios tireoidianos desempenham um papel crítico no metabolismo ósseo. Entretanto, dados relacionados ao efeito das alterações nos níveis destes hormônios na densidade mineral óssea mandibular e na progressão da doença periodontal são limitados. O objetivo do presente estudo foi avaliar, em ratos, a influência de diferentes níveis séricos dos hormônios tireoidianos: (1) na densidade radiográfica e na proporção dos componentes cortical e medular na mandíbula em comparação com a tíbia; e (2) na taxa de perda óssea resultante da periodontite induzida e na qualidade do osso alveolar, bem como no número de células com capacidade de reabsorção em sítios inflamados e não-inflamados por meio de análise histológica. Trinta e seis ratos Wistar machos foram aleatoriamente designados para os seguintes grupos: G1 (n=12) – controle saudável; G2 (n=12) – hipotireoidismo (1 g propiltiouracil / 1 l água); G3 (n=12) – hipertireoidismo (800 μg T_4 e 180 μg T_3 / 1 l água). Três meses após o início da indução das disfunções tireoidianas, as alterações nos níveis séricos de T_3 e T_4 total foram confirmadas por radioimunoensaio. Para indução da periodontite, as ligaduras foram posicionadas em um dos primeiros molares inferiores e, após 30 dias, os animais foram sacrificados. Radiografias digitais de uma hemimandíbula e uma tíbia por animal foram realizadas imediatamente e, em seguida, as hemimandíbulas foram processadas rotineiramente para obtenção de cortes descalcificados seriados. Os parâmetros radiográficos acessados foram: densidade da tíbia e da mandíbula e a proporção dos componentes cortical e medular destes ossos. Adicionalmente, os parâmetros histológicos avaliados foram: perda óssea induzida pela periodontite, densidade do osso alveolar e número de células positivas para fosfatase ácida tartarato resistente (TRAP), um marcador fenotípico de reabsorção óssea. A análise dos dados radiográficos demonstrou que a densidade óssea ($p<0,05$) e a proporção de osso cortical ($p<0,01$) foram influenciados na tíbia quando condições hormonais distintas foram

comparadas, com diminuição significativa nos parâmetros para o hipotireoidismo. Entretanto, na mandíbula não houve diferenças significativas entre os grupos para ambos parâmetros ($p>0,05$). Com relação aos dados histológicos, nos sítios com ligadura, o hipotireoidismo aumentou significativamente a perda óssea induzida pela periodontite ($p<0,05$) e o número de células TRAP-positivas na superfície linear da crista óssea ($p\leq0,01$). Não foram detectadas diferenças significantes entre os grupos na densidade óssea alveolar ($p>0,05$). Portanto, dentro dos limites do presente estudo, pode-se concluir que o osso mandibular parece ser menos sensível que a tíbia a mudanças radiográficas induzidas por alterações nos hormônios tireoidianos. Além disso, hipotireoidismo parece aumentar a perda óssea relacionada à periodontite experimental, em função do aumento no número de células com capacidade de reabsorção óssea.

Palavras-chave: triiodotironina, tiroxina, densidade mineral óssea, osso alveolar, periodontite.

Thyroid hormones play a critical role on bone metabolism. However, data regarding the effect of thyroid hormones alterations on mandibular bone density and on the progression of periodontal disease are limited. The aim of the present study was to evaluate, in rats, the influence of different serum levels of thyroid hormones on: (1) the bone mineral density and the proportion of cortical and cancellous components in mandibles compared to tibia by radiographic evaluation; and (2) the rate of periodontal bone loss resulting from ligature placement and on the quality of tooth-supporting alveolar bone, as well as on the number of resorbing cells on inflamed and non-inflamed sites by histologic analysis. Thirty-six male Wistar rats were randomly assigned to the following groups: G1 (n=12) – healthy (control); G2 (n=12) – hypothyroidism (1 g propylthiouracil / 1 l drinking water); G3 (n=12) – hyperthyroidism (800 μg T_4 and 180 μg T_3 / 1 l drinking water). Three months after the beggining of the induction of thyroid dysfunctions, total serum levels of T_3 and T_4 alterations were confirmed by radioimmunoassay. For periodontitis induction, ligatures were randomly placed around one of the first mandibular molars and, thirty days later, the animals were sacrificed. Digital radiographs were immediately taken from one tibiae and one hemimandible of each animal and, subsequently, the hemimandibles were routinely processed for serial decalcified sections. The radiographic parameters assessed were tibiae and mandibular bone density and the proportion of cortical and cancellous components in both bone types were assessed. In addition, the histologic parameter analyzed were periodontitis-related bone loss, quality of tooth-supporting alveolar bone, and the number of tartrate-resistant acid phosphatase (TRAP)-positive cells. Radiographic data analyses demonstrated that bone density ($p<0.05$) and the proportion of cortical bone ($p<0.01$) were influenced in the tibiae when distinct hormonal conditions were compared, with a significant decrease in the parameters for hypothyroidism. However, in the mandibles, there were no significant

differences among the groups neither for bone density nor for the proportion of cortical/cancellous components ($p>0.05$). Regarding histologic data, in the ligated sites, hypothyroidism significantly increased the bone loss resulting from ligature-induced periodontitis ($p<0.05$) and the number of TRAP-positive cells on the linear surface of bone crest ($p<0.05$). In addition, no significant differences were detected regarding the alveolar bone density ($p>0.05$). Therefore, within the limits of the present study it may be concluded that bone density and the proportion of cortical/cancellous bone in the mandibles seem to be less sensitive than tibia to the hormones' changes. Moreover, decreased serum levels of thyroid hormones may enhance periodontitis-related bone loss, as a function of an increased number of resorbing cells.

Key words: triiodothyronine, thyroxine, bone mineral density, alveolar bone, periodontitis.

Esta tese está baseada nos seguintes artigos científicos:

1. Feitosa DS, Menezes AV, Haiter-Neto F, Casati MZ, Sallum EA, Nociti Jr. FH, Toledo S. Thyroid hormones may influence bone density and cortical bone thickness in a site dependent manner. A radiographic study in rats. (not submitted)
2. Feitosa DS, Marques MR, Casati MZ, Sallum EA, Nociti Jr. FH, Toledo S. The influence of thyroid hormones on periodontitis-related bone loss and tooth-supporting alveolar bone: a histologic study in rats. J Periodont Res 2009;44:473-478.

SUMÁRIO

A glândula tireóide, localizada imediatamente abaixo da laringe, bilateral e anteriormente à traquéia, é uma das maiores glândulas endócrinas do corpo, normalmente pesando 15 a 20 g no adulto (Guyton & Hall, 2002, Silverthorn *et al.*, 2003). As células da tireóide produzem e secretam uma molécula de glicoproteína denominada tireoglobulina, a qual contém cerca de 70 aminoácidos tirosina, que constituem os principais substratos a se combinar com o iodo para formar 2 importantes hormônios, a triiodotironina e a tiroxina, comumente denominados T_3 e T_4 (Little, 2006). Os hormônios tireoidianos afetam os processos metabólicos do corpo. Estes hormônios desempenham papel crítico durante a embriogênese e a vida precoce e têm efeitos metabólicos profundos na vida adulta, incluindo mudanças no consumo de oxigênio e no metabolismo de proteínas, carboidratos, lipídeos e vitaminas (Oetting & Yen, 2007).

A manutenção dos níveis normais dos hormônios tireoidianos é controlada por um sistema de retroalimentação negativa mediado pelo eixo hipotálamo-hipófise-tireóide, por meio dos hormônios de liberação da tireotropina (TRH) e tireoestimulante (TSH). Sob condições normais, o TRH é liberado pelo hipotálamo em resposta a estímulos externos e estimula a secreção de TSH pela hipófise anterior, que, por sua vez, estimula a liberação de T_3 e T_4 pela tireóide (Guyton & Hall, 2002, Silverthorn *et al.*, 2003). Contudo, drogas, idade, alterações sistêmicas, além de desordens das glândulas tireóide e hipófise podem afetar a secreção de T_3 e T_4 para o sangue (Little, 2006). O aumento e a diminuição patológicos dos níveis séricos dos hormônios tireoidianos são denominados respectivamente de hiper e hipotireoidismo. Estas disfunções aparecem entre as alterações endócrinas mais comuns na população mundial, com prevalência que varia entre 0,9%-15,9% para o hipotireoidismo e 0,25%-7,3% para o hipertireoidismo (Bjoro *et al.*, 2000, Pontes *et al.*, 2002).

Além da sua ação no metabolismo basal, os hormônios tireoidianos desempenham um papel específico na manutenção da integridade do esqueleto.

Em condições normais, T_3 e T_4 ativam tanto a síntese quanto a degradação da matriz óssea (Bland, 2000). Estudos histomorfométricos in vivo mostraram que em condições de excesso dos hormônios tireoidianos, a atividade dos osteoblastos e osteoclastos está aumentada com predomínio da última. Como resultado, o metabolismo ósseo torna-se acelerado, favorecendo a reabsorção e a perda da massa óssea (Allain *et al.*, 1995). Em contraste, no hipotireoidismo, há uma marcante diminuição do metabolismo ósseo, caracterizada pela diminuição das atividades osteoblástica e osteoclástica (Eriksen *et al.*, 1985). No hipertireoidismo não-tratado, estes fenômenos se traduzem clinicamente em humanos pela diminuição da densidade óssea, bem como com o aumento no risco de fraturas em tais indivíduos (Jodar *et al.*, 1997, Siddiqi *et al.*, 1998, Vestergaard & Mosekilde, 2002). Além disso, apesar da influência do hipotireoidismo no tecido ósseo ser menos estudada, esta patologia também aparece associada com maior risco de fraturas (Vestergaard & Mosekilde, 2002).

Embora o mecanismo de ação dos hormônios tireoidianos no tecido ósseo ainda não esteja completamente elucidado, algumas evidências sinalizam o seu comportamento. Estudos *in vitro* mostraram que estes hormônios se ligam a receptores nucleares específicos exercendo uma ação direta nos osteoclastos e/ou indireta via osteoblastos (Britto *et al.*, 1994, Abu *et al.*, 1997). Os hormônios tireoidianos também parecem regular a expressão de uma série de genes relacionados à atividade dos osteoblastos. Em osteoblastos de ratos, T_3 induziu a atividade de fosfatase alcalina, bem como de osteocalcina e ainda a produção de colágeno (Rizzolli *et al.*, 1986, Oishi *et al.*, 1990). Adicionalmente, estes hormônios regulam certos fatores de crescimento e citocinas. T_3 induziu a expressão do fator de crescimento insulínico-1 (IGF-I) e de suas proteínas de ligação (IGFBP-2) em culturas primárias em ratos e em linhagens de células osteoblásticas, produzindo um aumento correspondente nos níveis de proteínas (Schimid *et al.,* 1992). Entre as citocinas, foi demonstrada regulação das interleucinas (IL) -6 e -8 (Siddiqi *et al.*, 1998).

Observações clínicas e experimentais têm apontado para uma característica peculiar dos hormônios tireoidianos, a promoção de uma resposta heterogênea, ou seja, sítio-específica, do esqueleto. Em uma avaliação *in vitro*, Milne *et al.* (1998) observaram que em osteoblastos cultivados de origem femoral, T_3 induziu a produção de mRNA de colágeno tipo I e de osteocalcina, mas praticamente não induziu a expressão do fator de crescimento insulínico-1 (IGF-I). Por outro lado, nos osteoblastos de origem vertebral apenas a expressão de mRNA da IGF-I foi marcadamente influenciada pelo tratamento com T_3. Uma série de estudos em animais confirmaram a diversidade de respostas ao hormônio tireoidiano. A administração de altas doses de T_4 por períodos de 3 a 20 semanas resultou em diminuição da densidade mineral no fêmur, mas não nas vértebras de ratos. Complementarmente, a expressão gênica de marcadores osteoblásticos e osteoclásticos foi investigada nestes animais. Aumento nos níveis de mRNA da osteocalcina, osteopontina, fosfatase alcalina, fosfatase ácida tartarato-resistente e histona (H4) foram identificados no fêmur, mas não nas vértebras dos ratos tratados com T_4 (Ongphiphadhanakul *et al.*, 1992, 1993, Suwanwalaikorn *et al.*, 1996). Finalmente, em humanos, Numbenjapon *et al.* (2007) mostraram por meio de tomografia computadorizada que apenas o componente cortical, mas não o medular, dos ossos de crianças e adolescentes com hipertireoidismo não-tratado sofreram diminuição da densidade. Estes achados levantam a questão de quando e por que alguns sítios são afetados preferencialmente pelo hipertireoidismo. Bem como se a diminuição dos níveis séricos dos hormônios tireoidianos têm uma tendência similar de influenciar de maneira heterogênea o tecido ósseo e quais mecanismos podem estar envolvidos. Com relação às potenciais explicações para a resposta heterogênea do esqueleto, além da expressão gênica de fatores relacionados a osteoblastos e osteoclastos e de fatores de crescimento serem sítio-específicas (Suwanwalaikorn *et al.*, 1996, Milne *et al.*, 1998), pode ser sugerido que uma diferente expressão da vitamina D e dos receptores de retinóide que formam complexos com os receptores dos hormônios tireoidanos poderiam regular a ação diferenciada destes hormônios (Williams *et al.*, 1994, Miura *et al.*,

2002). Ao passo que o impacto do hipotireoidismo em diferentes ossos precisa ainda ser caracterizado.

Como descrito, as medidas locais da densidade óssea em um sítio esquelético não necessariamente refletem a densidade óssea em outros sítios (Jacobs *et al.*, 1996). A ação dos hormônios tireoidianos, principalmente em ossos longos, tem sido documentada ao longo do tempo (Milne *et al.*, 1998, Ongphiphadhanakul *et al.*, 1992, 1993, Suwanwalaikorn *et al.*, 1996, Numbenjapon *et al.*, 2007). Entretanto, a despeito da alta prevalência das disfunções tireoidianas na população, apenas um estudo desenvolvido por Talaeipour *et al.* (2005) investigou o impacto da administração de altas doses de T_4 na densidade de ossos mandibulares e maxilares e parece não haver informações relativas à influência do hipotireoidismo nestes sítios. A importância da avaliação da densidade do osso mandibular tem sido discutida em odontologia por diversas razões: 1) como um fator indicativo da densidade óssea sistêmica determinado por radiografias intra-bucais convencionais ou panorâmicas, auxiliando no diagnóstico de patologias de ordem geral (Jeffcoat, 2005, Devlin & Horner, 2008); e, sobretudo, 2) pela hipótese da densidade óssea sistêmica e mandibular estarem relacionadas com o prognóstico de tratamentos que podem ser influenciados pela quantidade e qualidade do osso alveolar, especialmente reabilitações dentais, como próteses sobre implantes e próteses removíveis (Jaffin & Berman, 1991) ou pela associação entre a densidade óssea e a taxa de progressão da periodontite (Wactawski-Wende *et al.*, 2005).

A periodontite, por sua vez, é uma doença complexa cuja expressão envolve interações do biofilme com a resposta imuno-inflamatória do hospedeiro e alterações subsequentes na homeostasia do osso e do tecido conjuntivo (Kornman, 2008). Portanto, atualmente é reconhecido que o fenótipo da doença periodontal não resulta simplesmente do desafio microbiano traduzido por uma resposta padrão do hospedeiro. Fortes evidências destacam o papel de fatores de risco, como o tabagismo e o diabetes, como determinantes da severidade da doença periodontal (Bergström *et al.*, 2000, Campus *et al.*, 2005). Além destes,

vários outros fatores parecem modificar a expressão da doença, como, por exemplo, fatores genéticos, estresse e osteoporose (Kinane & Hart, 2003, Wactawski-Wende *et al.*, 2005, Peruzzo *et al.*, 2007). Na presença destes fatores modificadores, uma resposta exagerada do hospedeiro e/ou mecanismos de reparo prejudicados parecem levar a uma periodontite mais destrutiva (Kornman, 2008). Os hormônios tireoidianos têm importante papel na homeostasia do esqueleto por meio da sua ação sobre eventos moleculares como, por exemplo, no mecanismo do sistema osteoprotegerina / ativador do receptor do fator nuclear kB e seu ligante (OPG/RANK/RANKL) (Akalin *et al.*, 2002) e sobre fatores envolvidos na regulação óssea como IL-6 e IL-8 (Siddiqi *et al.*, 1998). Em virtude da influência de T_3 e T_4 em vias diretamente envolvidas na destruição periodontal, seria plausível a hipótese de que alterações nos níveis destes hormônios poderiam influenciar a progressão da destruição periodontal. Entretanto, não existem na literatura investigações a respeito do impacto destas condições sistêmicas na doença periodontal.

PROPOSIÇÃO

Avaliar a influência de diferentes níveis séricos dos hormônios tireoidianos:

1-Na densidade mineral óssea e nas proporções dos componentes cortical e medular da mandíbula comparativamente com a tíbia, por meio de análise radiográfica.

2-Na perda óssea resultante da periodontite experimental, na densidade do osso alveolar e na quantidade de células com capacidade de reabsorção óssea em sítios saudáveis e com periodontite, por meio de análise histológica.

.

CAPÍTULO 1

Thyroid hormones may influence bone density and cortical bone thickness in a site dependent manner. A radiografic study in rats.

Authors:

Daniela da Silva Feitosa [1]

Alynne Vieira de Menezes [2]

Francisco Haiter Neto[3]

Márcio Zaffalon Casati [4]

Enilson Antonio Sallum [5]

Antonio Wilson Sallum [5]

Francisco Humberto Nociti Júnior [5]

Sérgio de Toledo [5]

[1] PhD Program - Department of Prosthodontics and Periodontics – Division of Periodontics, Piracicaba Dental School, University of Campinas-UNICAMP, São Paulo, SP, Brazil.

[2] PhD Program - Department of Oral Diagnosis – Division of Dental Radiology, School of Dentistry at Piracicaba, State University of Campinas.

[3] Professor - - Department of Oral Diagnosis – Division of Dental Radiology, School of Dentistry at Piracicaba, State University of Campinas.

[4] Associate Professor - Department of Prosthodontics and Periodontics - Division of Periodontics, School of Dentistry at Piracicaba, State University of Campinas.

[5] Professor - Department of Prosthodontics and Periodontics - Division of Periodontics, School of Dentistry at Piracicaba, State University of Campinas.

Corresponding author:

Daniela da Silva Feitosa

Avenida Limeira 901, Areião, 13414-903, Piracicaba, São Paulo, Brazil.

Fax: 55-19-2106-5301

email: dsfeitosa@yahoo.com

ABSTRACT

Objective: This study aimed to radiographically evaluate the influence of thyroid hormones changes on the bone density and on the proportion of cortical and cancellous bone in tibia and mandibles of rats.

Methods: Thirty-six male Wistar rats were randomly assigned to the following groups: G1 (n=12) – healthy (control); G2 (n=12) – hypothyroidism; G3 (n=12) – hyperthyroidism. Three months after the induction of thyroid dysfunctions, total serum levels of T_3 and T_4 were assessed by radioimmunoassay. The animals were sacrificed after 2 months and digital radiographs were immediately taken from one tibiae and hemimandible in each animal, and the bone density and the proportion of cortical/cancellous components in both bone types were assessed by means of a software.

Results: Data analyses demonstrated that bone density ($p<0.05$) and the proportion of cortical bone ($p<0.01$) were influenced in the tibia when distinct hormonal conditions were compared, with a significant decrease in the parameters for hypothyroidism. However, in the mandibles, there were no significant differences among the groups neither for bone density nor for the proportion of cortical bone ($p>0.05$).

Conclusion: Within the limits of the present study, it may be concluded that bone density and the proportion of cortical/cancellous bone in the mandibles seems to be less sensitive than tibia to hormones' changes.

INTRODUCTION

The skeleton is a highly metabolically active organ that undergoes continuous remodeling throughout the life. This remodeling is necessary both to maintain structural integrity of the skeleton and to subserve its metabolic functions as a storehouse of calcium and phosphorus (Raisz, 1999). Adaptation of the material composition and structure of bone to prevailing loads is carried out by the cellular machinery of bone remodeling, that includes hormones, cytokines, growth factors, vitamins and minerals (Allori, 2008, Martin & Seeman 2008). Abnormalities in the rate and balance of bone remodeling play a pivotal role in the emergence of bone fragility and may be caused by disease, hormonal imbalance and excessive exposure to risk factors, as well as abnormalities in the cellular machinery of bone (Chavassieux et al., 2007, Seeman, 2008).

Thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4), are some of the several hormones that exert a balancing effect on bone (Talaeipour et al., 2005). These hormones may act on bone cells by influencing target genes via specific nuclear receptors (Abu et al., 1997). Thyroid hormones modulate alkaline phosphatase, osteocalcin, collagen and certain growth factors and cytokines involved in bone remodeling with respect to new bone formation (Sato et al., 1987, Milne et al., 1998, Kim et al., 1999). In vivo, the effects of T_3 and T_4 on bone are also well documented, however the response to thyroid hormones varies depending on the skeletal sites (Bland, 2000). Administration of L-thyroxine to adult rats preferentially affects femoral but not vertebral bone density (Suwanwlaikorn et al. 1996). And in humans, in a large population based study, an increased risk of femur fractures in patients with hyperthyroidism in contrast with an overall increase in fracture risk in patients with hypothyroidism were reported (Vestergaard & Mosekilde, 2002). Finally, Nunbenjapon et al. (2007) observed that cancellous component of bone seemed to be less sensitive than cortical one to thyroid hormones dysfunction in children and adolescents.

In summary, local bone density measurements at one site of the skeleton do not necessarily reflect bone density at another site (Jacobs et al., 1996). However, there is scarce information on whether systemic bone density influenced by hormonal conditions, in special thyroid hormones dysfunctions, is associated to mandibular bone density. Therefore, the purpose of the present study was to radiographically evaluate in rats the influence of different serum levels of T_3 and T_4 on the bone mineral density and the percentage of cortical and cancellous bone on mandible and tibiae to test the hypothesis that both bone types have a similar pattern of sensitivity.

MATERIALS AND METHODS

Animals

The study included 36 male Wistar rats, aged 60 days and weighing an average of 194.44 ± 27.52 g at study onset. The animals were kept in plastic cages with access to food and water ad libitum. Prior to the thyroid dysfunctions induction, all animals were allowed to acclimatize to the laboratory environment for a period of 5 days. The protocol was approved by University of Campinas Institutional Animal Care and Use Commitee.

Experimental design

At the beginning of the study, the animals were randomly assigned to one of the following experimental groups: G1 (n=12): control / healthy animals; G2 (n=12): hypothyroidism, which was induced by the ingestion of a solution with propylthiouracil (Propilracil® 100mg, Biolab Sanus Farmacêutica Ltda., Taboão da Serra, SP, Brazil) (1 g / 1 l of drinking water), an anti-thyroid drug which blocks thyroid hormone synthesis; and G3 (n=12): hyperthyroidism, which was induced by the ingestion of sodium l-thyroxine (T_4) (Puran® T_4 200 µg, Sanofi-Synthelabo Ltda., Rio de Janeiro, RJ, Brazil) and sodium triiodothyronine (T_3) (Sodium Triiodothyronine 90 µg, Drogal Ltda., Piracicaba, SP, Brazil) (800 µg T_4 and 180 µg T_3 / 1 l of drinking water). The animals were continuously administered with the

drugs for hormone changes during five months, the whole experimental period, without interruption, as previously reported (Feitosa *et al.*, 2008). Figure 1 schematically illustrates the experimental design adopted.

Biochemical serum analyses

Three months after the beginning of the study and, therefore, induction of hormone changes, blood samples were collected for the assessment of total serum levels of T_3 and T_4 by radioimmunoassay (RIA; Active Triiodothyronine (T_3) RIA DSL-3100 and Active Thyroxine (T_4) RIA DSL-3200, Diagnostic System Laboratories Inc., Webster, Texas, United States), according to the manufacturer's instructions (Girardi *et al.*, 2000). This protracted period of induction of dysfunctions was chosen to obtain chronic hormonal alterations.

Radiographic analysis

Immediately after sacrifice, the hemimandibles and the tibia were removed and radiographed using a GE 1000® X-ray unit (General Electric Co., Milwaukee, WI, USA) at 60.0 kVp, 10.0 mA , a 40.0 cm focus-receptor distance and with an exposure time of 0.3 seconds. An acrylic device was used to hold the phantom, X-ray beam indicator device and image plate in a reproducible relationship. The DenOptix® system (Denstply International/Gendex® Dental X-ray Division, Des Plaines, IL, USA), a phosphor plate technique, was used for image recording. The exposed phosphor plates were scanned and the resolution was set at 300dpi.

An aluminium step wedge of 2-16 mm thickness was used, with increments of 2 mm. To allow the analysis of bone density, the images were then imported to EMAGO®/Advanced 3.43 software (Oral Diagnostic Systems, Amsterdam, The Netherlands). The reference point was determined as the mean point of the total length of the tibiae and the mean point of mandibular ramus (Figure 2A-B). A blinded and calibrated examiner performed five measurements, in order to scan the bone area at the level of a previously determined reference point. Subsequently, the measurement of bone density was compared with the step wedge standard to provide a unit for quantifying bone density, which was

expressed in milimeters aluminium equivalence (mm Al eq). (César-Neto et al., 2005a, b).

In addition, in the same radiographs the percentages of the cortical and cancellous bone of the tibiae and the mandibles were evaluated by the use of an image analyzer (Image Tool; University of Texas Health Science Center, San Antonio, TX, USA). With the aim of calculating these parameters, in the tibiae, the thickness of cortical bone (C1 + C2) and the total bone (T) were assessed in five equally distant regions to scan the whole length of the tibiae and the following formula was applied, C%= (C1 + C2)/T x 100, as suggested in a previous report (Serakides et al., 2004). In the mandibular ramus, the area of cortical bone (C) and total bone (T) were also assessed and the formula used was C%= C/T x 100. This measurement was repeated five times and the mean was used. Figure 3A-B illustrates the measurements of radiographs.

Statistical analyses

Initially, a total of 10 radiographs of mandibles and 10 radiographs of tibia were selected to obtain the intra-observer calibration. The designated examiner measured the parameters twice, within one week between the evaluations. Intra-observer variability was assessed with intraclass correlation calculated to the parameters of bone density; percentage of cortical bone in the tibiae; and percentage of cortical bone in the mandibular ramus, resulting in 99%, 99% and 97% reproducibility, respectively.

The hypothesis that there were no differences among the groups with respect to the hormone serum levels (T_3 and T_4) and radiographic parameters (e.g., bone density and percentages of cortical and cancellous bone for tibiae and mandible) were tested by the one-way analysis of variance – ANOVA (α=0.05). If statistical differences were detected, a pairwise multiple comparison procedure, Tukey test, was used with alpha=0.05. The SAS 9.01 software (Release 9.1, 2003, SAS institute Inc., Cary, NC, USA) was used to perform the statistical analyses.

RESULTS

Clinical observations and biochemical markers

Macroscopic examination during autopsies suggested the successful induction of thyroid dysfunctions. Thyroid glands in control and hyperthyroidism groups were larger and pink colored, while in hypothyroidism the glands were thin and anemic due to reduced activity. The total serum levels of T_3 and T_4 confirmed hormone changes and are summarized in Table 1. The serum levels of T_3 and T_4 were higher in hyperthyroidism ($p<0.05$) and decreased in hypothyroidism ($p<0.05$), as compared to the control group.

Radiographic analyses

Data analyses did not show any significant difference regarding bone density of the tibiae when hypothyroidism and hyperthyroidism were compared to the control one ($p>0.05$). However, when distinct hormonal conditions, e.g. hypothyroidism versus hyperthyroidism were compared, a significantly reduced bone density was observed to hypothyroidism ($p<0.05$) (Figure 4A). Moreover, hypothyroidism group showed a decreased percentage of cortical bone compared to hyperthyroidism ($p<0.01$) (Figure 4B).

The same trend was not detected when the mandibular radiographic parameters were analysed, there were no significant difference among the groups neither for the bone density ($p>0.05$) nor for the percentage of cortical bone ($p>0.05$) (Figure 5A-B).

DISCUSSION

The present study was designed to radiographically evaluate the influence of serum thyroid hormones levels on the bone density and on the percentage of cortical and cancellous components of bone on the mandible, an irregular bone, in comparison to the tibiae, a long bone. With this purpose, changes on thyroid hormone levels (hypothyroidism and hyperthyroidism) were successfully induced

by drug ingestion and were detected by biochemical analyses of T_3 and T_4 as previously reported (Feitosa et al., 2008). Furthermore, the results obtained showed a significant decrease on bone density of the tibiae for hypothyroidism group when compared to hyperthyroidism. It may have occurred as a function of the significant decrease in the proportion of cortical bone in the hypothyroidism group.

Thyroid hormones clearly play a role in the normal bone development (Bland, 2000). Alterations on bone density (BMD) influenced by thyroid dysfunctions have been extensively discussed in the literature. On long bones of rats, such as femur and tibiae, bone density was influenced after the administration of high doses of T_4 (Yamamoto et al., 1993, Suwanwalaikorn et al., 1996). Nevertheless, the response to thyroid hormones seems to vary depending on the skeletal sites, since the same studies that showed a significant response on long bones, were unable in detecting this influence in other sites, e.g. lumbar vertebra and hip (Yamamoto et al., 1993, Suwanwalaikorn et al., 1996). Otherwise, the impact of thyroid hormones on mandibular bone density was scarcely described. In dentistry, the assessment of this information may be considered as useful or even necessary in many clinical situations. Applications include diagnosis of oral and systemic diseases, implant planning, therapeutic evaluation and follow-up (Nackaerts et al., 2007). According to the data obtained in the present study, mandibular bone seemed to be less sensitive to hormone changes whereas the radiographic analyses were developed. Both parameters, radiographic bone density and percentages of cortical and cancellous bone, did not show significant differences among the groups.

Defining the molecular mechanisms by which thyroid hormone acts in bone is a complex task because multiple forms of the thyroid receptor (TR) exist, with specific roles for each of these isoforms not yet determined definitively in any tissue. Firstly, it was suggested that the predominance of a specific TR isoform at either of the skeletal sites could provide an explanation for the different thyroid hormone responses (Milne et al., 1998). However, definite conclusions were not

drawn until now and the reason by which thyroid hormones seem to influence differently certain sites (long/irregular bone and cortical/cancellous bone) is already unclear. Miura et al. (2002) and Bassett et al. (2007) showed different responses to thyroid hormones deficiency between bones characterized by intramembranous ossification, e.g. skull, compared to others characterized by endochondral ossification, such as tibiae. These studies showed that TRs promote chondrocyte differentiation and predominantly stimulate endochondral osssification. Other regulatory factors that may be responsible for this skeletal site specific heterogeneity have been documented. T_3 regulates target gene transcription via the TRs which can form complexes with retinoic acid, dihydroxyvitamin D, or retinoic acid receptors to modify T_3 responsiveness and there may exist difference in the thyroid receptor heterodimerization pattern in tibiae versus mandibular osteoblasts (Williams et al., 1994). And finally, heterogeneity of thyroid hormone actions may be present due to postreceptor modifications, such as the modulation of the production of IL-6 and IL-8 (Siddiqi et al., 1998), insulin like growth factor-1, insulin like growth factor binding proteins (Milne et al., 1998), c-fos protein (Kanatani et al., 2004) and RANKL-RANK interaction (Kanatani et al. 2004).

In addition to the knowledge about the mechanisms that govern thyroid hormones action on bone tissue, it must be taken into consideration that the radiographic technique presented in this study may be related to the slight differences observed in the tibiae density and the absence of significant differences in the parameters analyzed for the mandible. Generally, 30-60% of regional bone destruction must have occurred for a change to be detected on radiographs and slight changes on bone density are not visualized in conventional radiographs (Talaeipour et al., 2005). However, it has been suggested that radiographic method using the aluminium step wedge minimize this limitation and may be used based on the evidence that accuracy and repeatability were proven to be excellent for the assessment of bone density (Nackaerts et al., 2006, 2007). Therefore, further animal and human studies should be considered in order to specifically address the effect of thyroid hormone changes on mandibular bone by means of gold

standard techniques such as dual energy x-ray absorptiometry or computerized tomography.

Many studies have attempted to address the association between systemic bone density and oral bone density. The present study was the first to test the hypothesis of whether dysfunctions in the serum levels of thyroid hormones which influence long bones, in agreement with several findings in the literature, may promote a similar impact on the mandibular bone. Within the limits of this study, thyroid hormones may influence the density and the proportion of cortical and cancellous bone in the tibiae, but mandible seems to be less sensitive to the hormones' changes.

REFERENCES

1. Raisz LG. Physiology and pathophysiology of bone remodeling. Clinical Chemistry 1999;45:1353-1358.

2. Allori AC, Sailon AM, Warren SM. Biological basis of bone formation, remodeling, and repair – part I: biochemical signaling molecules. Tissue Eng 2008;14(3):259-273.

3. Martin TJ, Seeman E. Bone remodeling: its local regulation and the emergence of bone fragility. Best Pract Res Clin Endocrinol Metab 2008;22(5):701-22.

4. Chavassieux P, Seeman E, Delmas PD. Insights into material and structural basis of bone fragility from diseases associated with fractures: how determinants of the biochemical properties of bone are compromised by disease. Endocr Rev 2007; 28(2):151-164.

5. Seeman E. Bone quality: the material and structural basis of bone strength. J Bone Miner Metabol 2008;26:1-8.

6. Talaeipour AR, Shirazi M, Kheirandish Y, Delrobaie A, Jafari F, Dehpour AR. Densitometric evaluation of skull and jaw bones after administration of thyroid hormones in rats. Dentomaxillofac Radiol 2005;34:332-336.

7. Abu EO, Bord S, Horner A, Chatterjee VKK, Compston JE. The expression of thyroid hormone receptors in human bone. Bone 1997:21(2):137-142.
8. Sato K, Han DC, Fujii Y, Tshushima T, Shizume K. Thyroid hormone stimulates alkaline phosphatase in cultured rat osteoblastic cells (ROS 17/2.8) through 3,5,3'triiodo-L-thyronine nuclear receptors. Endocrinology. 1987; 120: 1873-81.
9. Milne M, Kang M-I, Cardona G, Quail JM, Braverman LE, Chin WW, Baran DT. Expression of multiple thyroid hormone receptor isoforms in rat femoral and vertebral bone and in marrow osteogenic cultures. J Cell Biochem 1999;74:684-693.
10. Kim C-H, Kim H-K, Shong YK, Lee K-U, Kim GS. Thyroid hormone stimulates basal and interleukin (IL)-1-induced IL-6 production in human boné marrow stromal cells: A possible mediator of thyroid hormone-induced bone loss. J Endocrinol. 1999; 160:97-102.
11. Bland R. Steroid hormone receptor expression and action bone. Clin Sci 2000;98:217-240.
12. Suwanwalaikorn S, Ongphiphadanakul B, Braverman LE, Baran DT. Differential responses of femoral and vertebral bones to long-term excessive l-thyroxine administration in adults rats. Eur J Endocrinol 1996; 134: 655-659.
13. Vestergaard P, Mosekilde L. Fractures in patients with hyperthyroidism and hypothyroidism: a nationwide follow-up study in 16.249 patients. Thyroid 2002;12:411-419.
14. Numbenjapon N, Costin G, Gilsanz V, Pitukcheewanont P. Low cortical bone density measured by computed tomography in children and adolescents with untreated hyperthyroidism. J Pediatr 2007;150:527-530.
15. Jacobs R, Ghyselen J, Koninckx P, van Steenberghe D. Long term bone mass evaluation of mandible and lumbar spine in a group of women receiving hormone replacement therapy. Eur J Oral Sci 1996;104:10-16.
16. Feitosa DS, Bezerra BB, Ambrosano GMB, et al. Thyroid hormones may influence cortical bone healing around titanium implants. A histometric study in rats. J Periodontol 2008; 79: 881-887.

17. Girardi AC, Titan SM, Malnic G, Rebouças NA. Chronic effect of parathyroid hormone in NHE3 expression in rat renal proximal tubules. Kidney Int 2000; 58: 1623-1631.

18. César-Neto JB, Benatti BB, Manzi FR, Salum EA, Sallum AW, Nociti FH Jr. The influence of cigarette smoke inhalation on bone density. A radiographic study in rats. Braz Oral Res 2005;19(1):47-51.

19. César-Neto JB, Benatti BB, Haiter FN, Sallum AW, Salum EA, Nociti FH Jr. Smoking cessation may present a positive impact on mandibular bone quality and periodontitis-related bone loss: a study in rats. J Periodontol 2005;76:520-525.

20. Serakides R, Nunes VA, Ocarino NM, Nascimento EF. Bone effects of the association hyperthyroidism-castration of adult famale rats. Arq Bras Endocrinol Metab (in Portuguese) 2004;48:875-884.

21. Bland R. Steroid hormone receptor expression and action bone Clin Sci. 2000;98:217-40.

22. Yamamoto M, Balena R, Markatos A, Rodan GA. The effects of the aminobisphosphonate alendronate on thyroid hormone induced osteopenia in rats. Calcif Tissue Int 1993;53:278-82.

23. Nackaerts O, Jacobs R, Horner, Zhao F, Lindh C, Karayianni K, Van der Stelt P, Pavitt S, Devlin H. Bone density measurements in intra-oral radiographs. Clin Oral Invest 2007;11:225-229.

24. Milne M, Kang MI, Quail JM, Baran DT. Thyroid hormone excess increases insulin-like growth factor I transcripts in bone marrow cell cultures: divergent effects on vertebral and femoral cell cultures. Endocrinology 1998;139:2527-2534.

25. Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, Ozasa A, Takao K. Thyroid hormones promote chondrocyte differentiation in mouse ATDC5 cells and stimulate endochondral ossification in fetal mouse tibias through iodothyronine deiodinases in the growth plate. J Bone Miner Res 2002; 17(3):443-54.

26. Bassett JHD, Nordström K, Boyde A, Howell PGT, Kelly S, Vennström B, Williams GR. Thyroid status during skeletal development determines adultbone structure and mineralization. Mol Endocrinol 2007;21(8):1893-1904.
27. Williams GR, Bland R, Sheppard MC. Characterization of thyroid hormone (T3) receptors in three osteosarcoma cell lines of distinct osteoblast phenotype: interactions among T3, vitamin D, and retinoid signaling. Endocrinology 1994;135:2375-2385.
28. Siddiqi A, Burrin JM, Wood DF, Monson JP. Tri-iodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. J Endocrinol 1998;157:453-461.
29. Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. J Cel Physiol 2004;201:17-25.
30. Talaeipour AR, Shirazi M, Kheirandish Y, Delrobaie A, Jafari F, Dehpour AR. Densitometric evaluation of skull and jaw bones after administration of thyroid hormones in rats. Dentomaxillofac Radiol 2005;34:332-36.
31. Nackaerts O, Jacobs R, Pillen M, Engelen L, Gijbels F, Devlin H, Lindh C, Nicopoulou-Karayianni K, Van der Stelt P, Pavitt S, Horner K. Accuracy and precision of a densitometric tool for jaw bone. Dentomaxillofacial Radiol 2006;35:244-248.

ACKNOWLEDGMENTS

Dr. Feitosa gratefully thanks the The National Council for Scientific and Technological Development – CNPq, Brasília, DF, Brazil, for their financial support.

TABLE

Table 1. Means and standard deviation of serum levels of triiodothyronine (ng/dl) and thyroxine (µg/dl) achieved in each experimental group.

Group	Triiodothyronine (ng/dl)	Thyroxine (µg/dl)
Control	111.53 ± 17.41 B	2.64 ± 0.58 B
Hypothyroidism	78.07 ± 12.11 C	1.28 ± 0.17 C
Hyperthyroidism	182.07 ± 31.40 A	4.18 ± 0.88 A

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

FIGURES

Figure 1. Illustration of the experimental design.

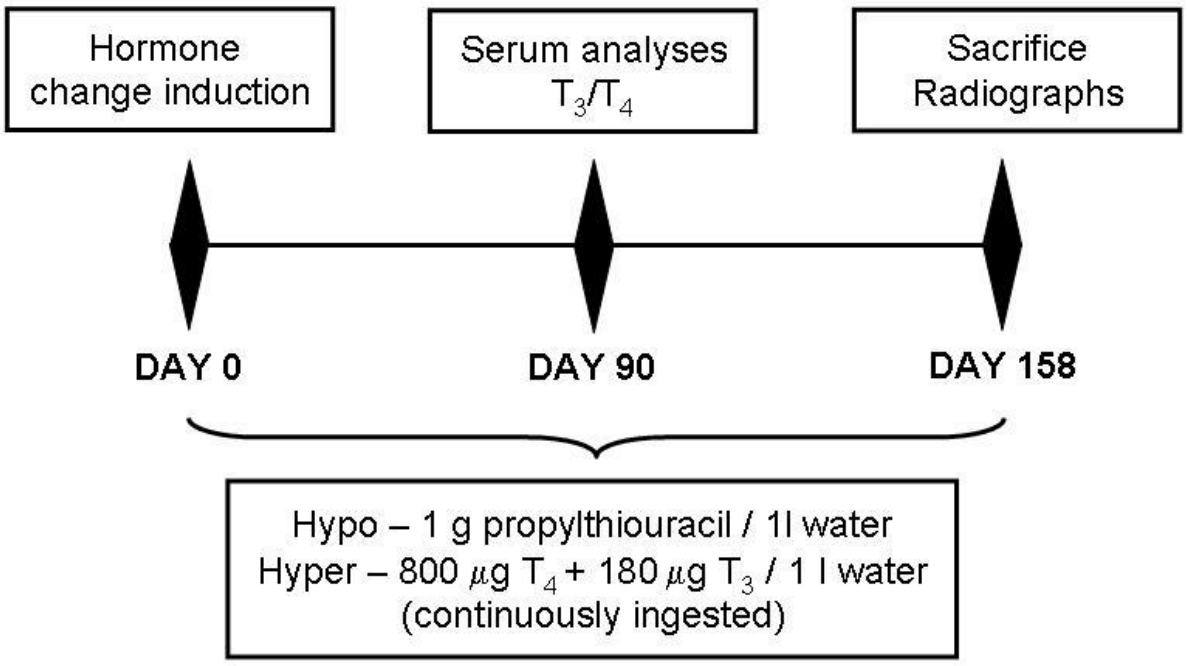


Figure 2. Radiographic images illustrating the tibiae (A) and mandibular (B) areas where photodensitometric measurements were performed.

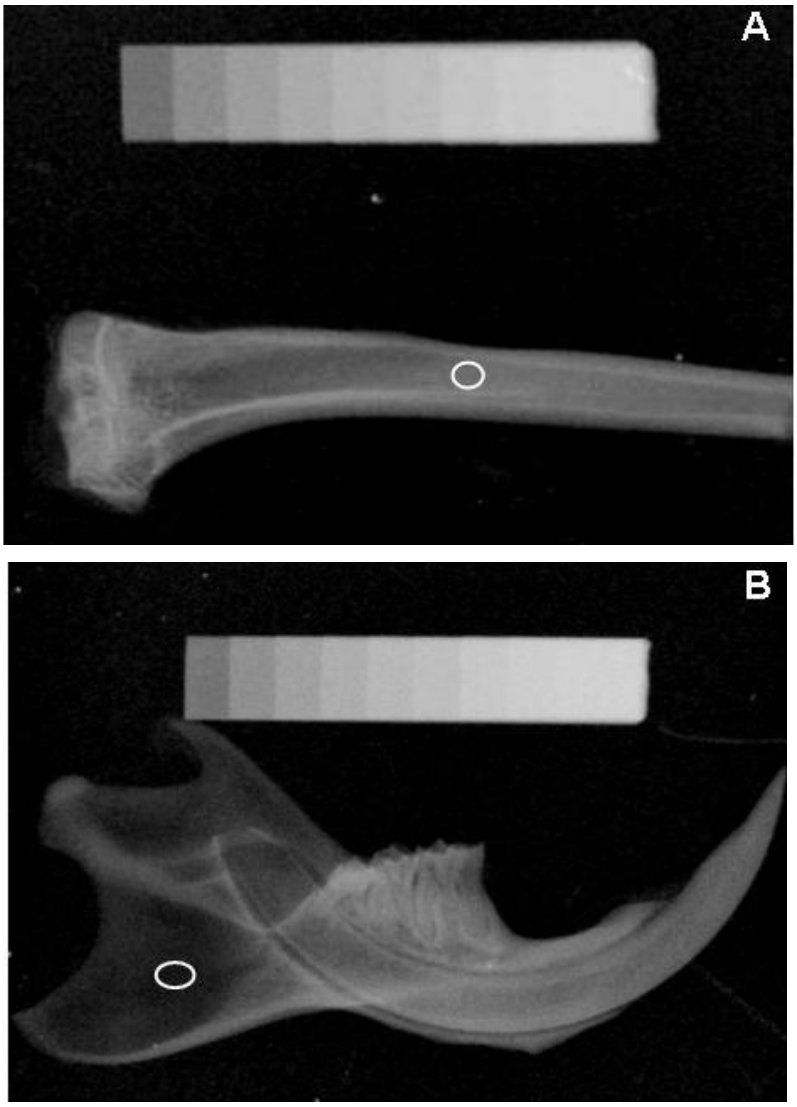


Figure 3. Radiographic images illustrating the parameters used to calculate the percentages of cortical and cancellous components of bone. In the tibiae (A), C1 and C2 = thickness of cortical components; and T = thickness of the total bone. In the mandibles (B), C = cortical area of the ramus, and T = area of the total ramus.

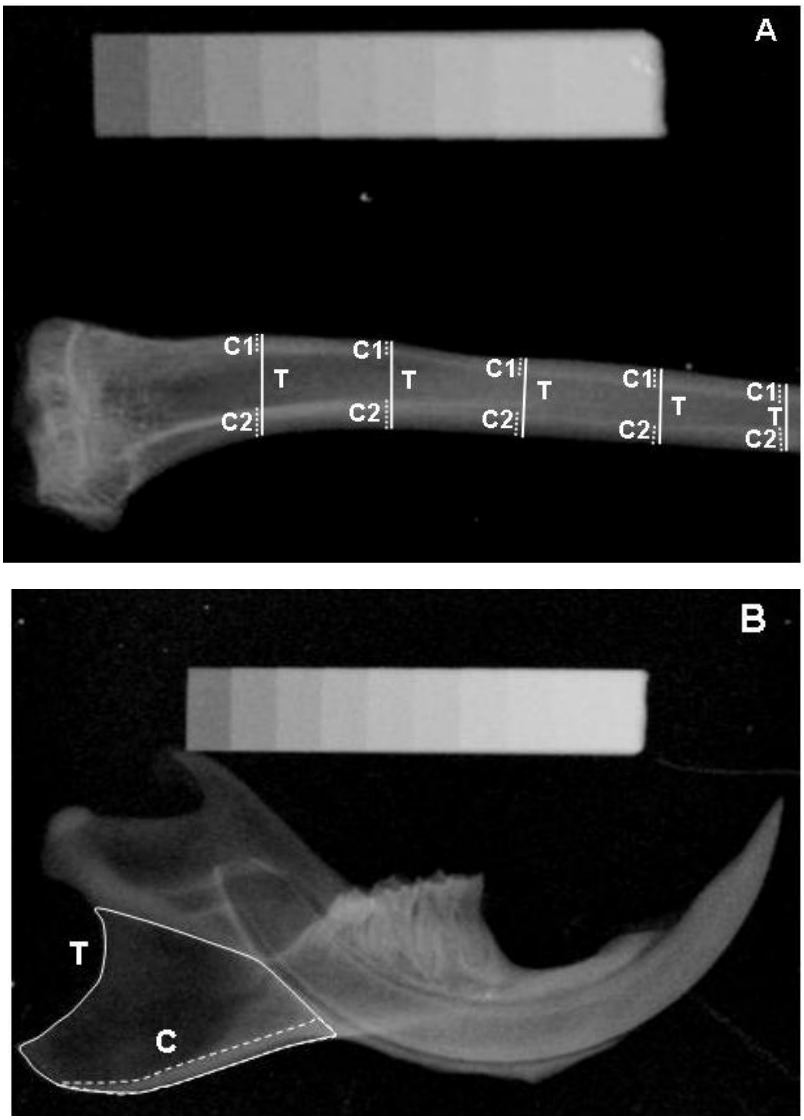


Figure 4. The illustration graphically represents the means and the standard deviation of the study parameters achieved for control, hypothyroidism and hyperthyroidism groups. A. Bone density of tibiae (mm Al eq) B. Percentage of cortical and cancellous bone of tibiae (%).

Means followed by different letters indicate significant difference (α = 0.05) by the one-way analysis of variance (ANOVA) and Tukey's test.

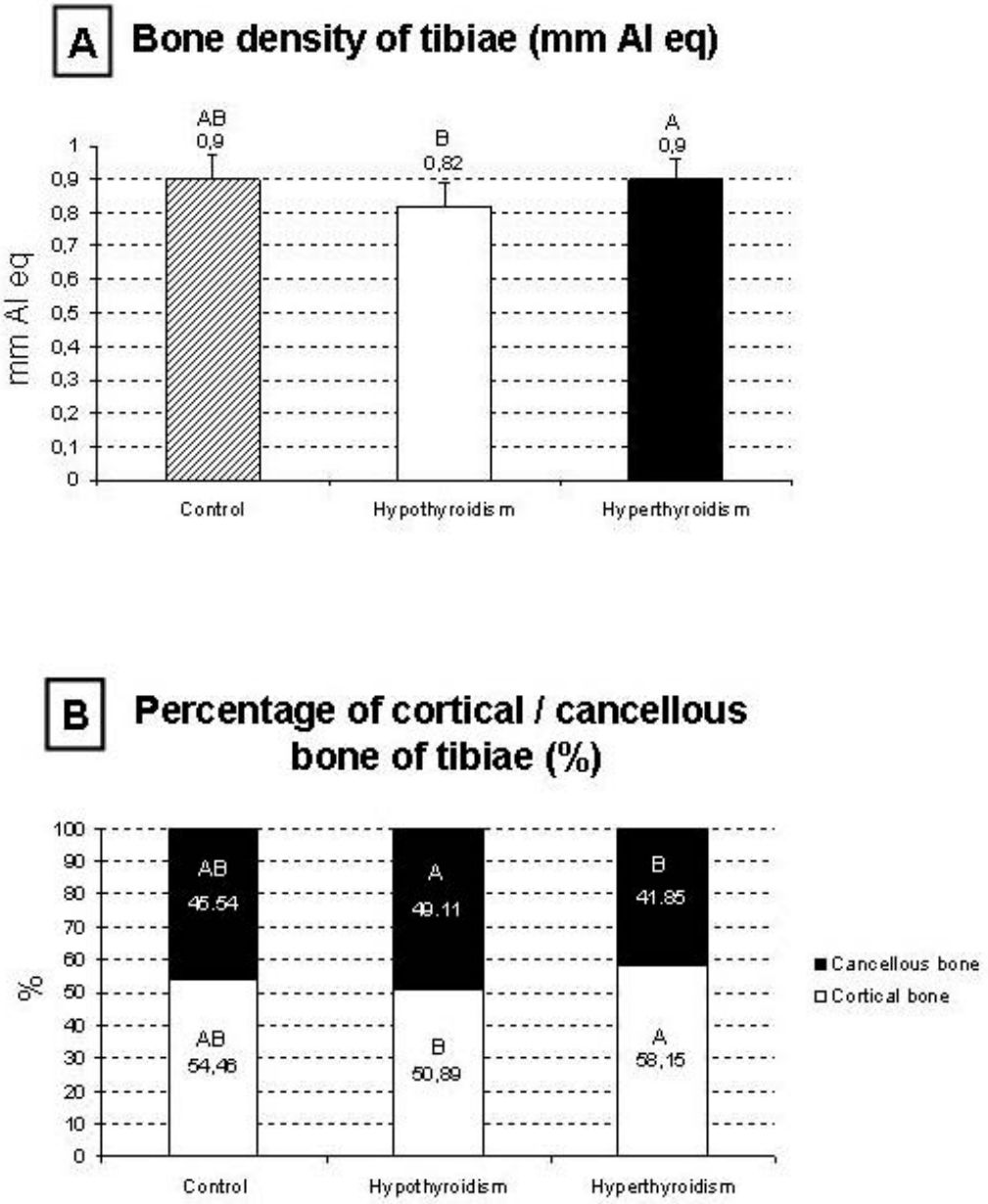
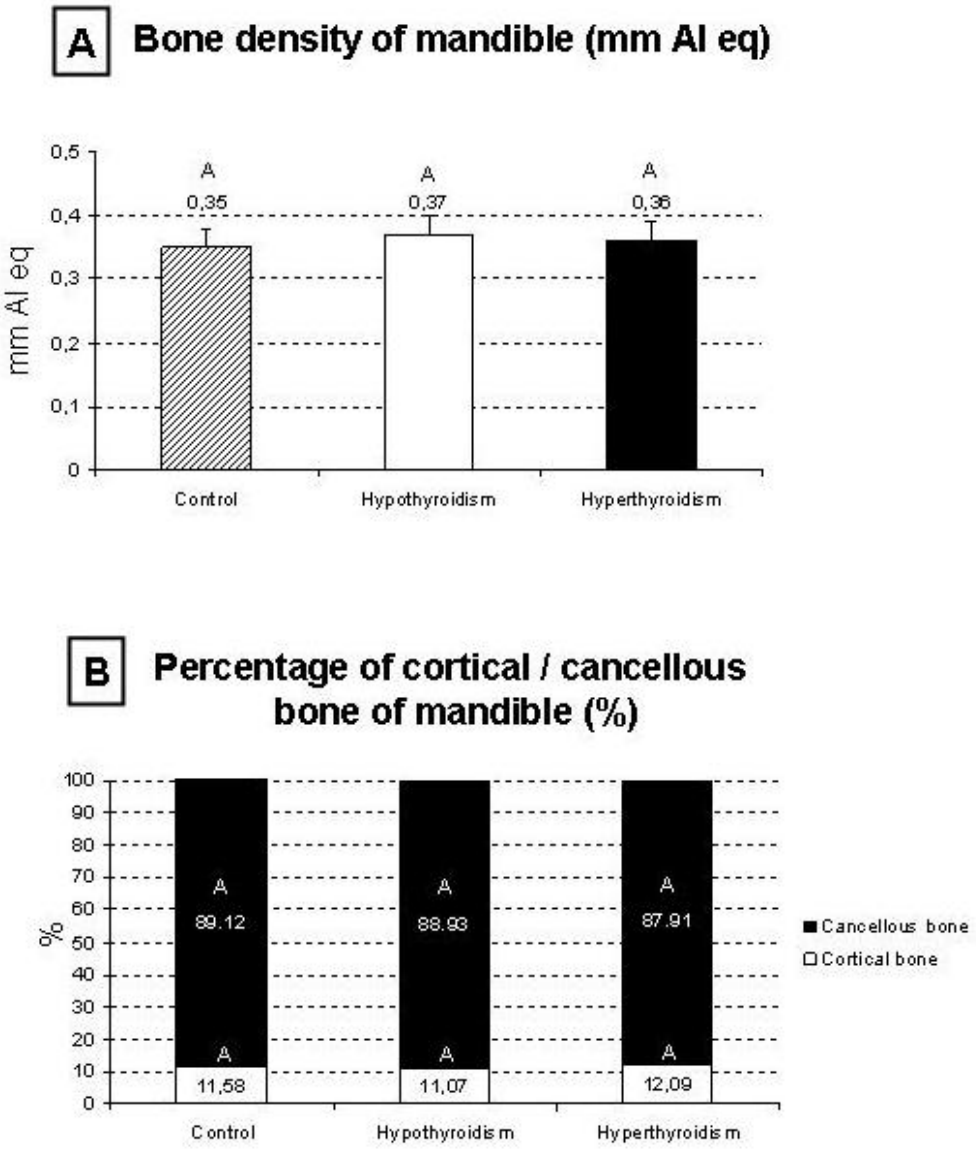


Figure 5. The illustration graphically represents the means and the standard deviation of the study parameters achieved for control, hypothyroidism and hyperthyroidism groups. A. Bone density of mandible (mm Al eq) B. Percentage of cortical and cancellous bone of mandible (%).

Means followed by similar letters indicate there is no significant difference (α = 0.05) by the one-way analysis of variance (ANOVA) and Tukey's test.



The influence of thyroid hormones on periodontitis-related bone loss and tooth-supporting alveolar bone: a histologic study in rats

Authors:

Daniela da Silva Feitosa[1]

Marcelo Rocha Marques[2]

Márcio Zaffalon Casati[3]

Enilson Antonio Sallum[4]

Francisco Humberto Nociti Jr[4]

Sérgio de Toledo[4]

[1]PhD Program - Department of Prosthodontics and Periodontics – Division of Periodontics, School of Dentistry at Piracicaba, State University of Campinas.

[2]PhD - Department of Morphology – Division of Histology, School of Dentistry at Piracicaba, State University of Campinas.

[3]Associate Professor - Department of Prosthodontics and Periodontics - Division of Periodontics, School of Dentistry at Piracicaba, State University of Campinas.

[4]Professor - Department of Prosthodontics and Periodontics - Division of Periodontics, School of Dentistry at Piracicaba, State University of Campinas.

Running tittle: Thyroid hormones and periodontal tissues

Corresponding author:

Daniela da Silva Feitosa

Avenida Limeira 901, Areião, 13414-903, Piracicaba, São Paulo, Brazil.

Fax: 55-19-2106-5301

email: dsfeitosa@yahoo.com / danielafeitosa@fop.unicamp.br

Key Words: periodontitis, alveolar bone, triiodothyronine, thyroxine.

ABSTRACT

Background and objective: The aim of the present study was to histologically evaluate, in rats, the influence of thyroid hormones on the rate of periodontal bone loss resulting from ligature placement and on the quality of tooth-supporting alveolar bone.

Methods: Thirty-six male Wistar rats were randomly assigned to the following groups: G1 (n=12) – healthy (control); G2 (n=12) – hypothyroidism; G3 (n=12) – hyperthyroidism. Once alterations were confirmed by total serum levels of T_3 and T_4, ligatures were randomly placed around one of the first mandibular molars. Thirty days later, the animals were sacrificed and the specimens routinely processed for serial decalcified sections. The parameters assessed were periodontitis-related bone loss, quality of tooth-supporting alveolar bone, and the number of tartrate-resistant acid phosphatase (TRAP)-positive cells, a marker of bone resorption.

Results: In the ligated sites, intergroup analysis revealed that hypothyroidism significantly increased the bone loss resulting from ligature-induced periodontitis ($P = 0.02$) and the number of TRAP-positive cells on the linear surface of bone crest ($P = 0.01$). In addition, no significant differences were detected regarding the quality of the bone ($P = 0.24$) or the number of TRAP-positive cells in the area of the interradicular bone among the groups for ligated teeth ($P = 0.17$).

Conclusions: It may be concluded that decreased serum levels of thyroid hormones may enhance periodontitis-related bone loss, as a function of an increased number of resorbing cells, whereas the tooth-supporting alveolar bone seems to be less sensitive to alterations in hormone levels.

INTRODUCTION

Periodontal diseases are infections characterized by an imbalance between bacterial challenging and host response (1). The host reaction to microbial insults involves recruitment of inflammatory cells, production of prostaglandins and cytokines, elaboration of lytic enzymes and activation of osteoclasts, leading to alveolar bone resorption and attachment loss (2). Although the dental biofilm is the primary cause of periodontal disease, *in vivo* studies have demonstrated that systemic factors may play an important role in its initiation and progression (3,4).

Triiodothyronine (T_3) and thyroxine (T_4) are hormones secreted by the thyroid gland, and have been shown to be fundamental for normal bone turnover (5). Decreased or increased levels of these hormones may be pathologically secreted to the blood, characterizing the conditions known as hypothyroidism and hyperthyroidism, respectively (6). In hypothyroidism, for instance, bone turnover is slow, bone growth and maturation are retarded in childhood and adults tend to exhibit higher than normal bone density, accompanied with increased fracture risk (7,8). On the other hand, hyperthyroidism is associated with accelerated bone maturation, high bone turnover, low bone mass and an increased life-time risk for fractures (7-9). Furthermore, recent findings reported the presence of thyroid hormones receptors (TRs) in osteoblasts, suggesting a direct skeletal effect of these hormones (10). It has been proposed that thyroid hormones have an important role in controlling bone resorption through their action on the osteoprotegerin (OPG) and receptor activator of nuclear factor Kb ligand (RANKL) mechanism (11), and on bone regulating factors such as interleukin-6 (IL-6) and interleukin-8 (IL-8) (12,13). Since an impact on bone is a prominent feature in periodontal disease, thyroid hormone level alterations may be suggested to be a modulating factor in periodontal disease, such as other systemic conditions, such as smoking and diabetes (14,15).

To date, no information is available on the impact of changes in T_3 and T_4 serum levels on the bone loss resulting from periodontitis and/or the quality of the

alveolar bone. Thus, the present study was designed to evaluate the influence of thyroid hormones on the bone loss resulting from experimental periodontitis and on the quality of tooth-supporting alveolar bone around teeth by histometric analysis. Additionally, we aimed to assess the impact of these hormones on the number of tartrate-resistant acid phophatase (TRAP)-positive cells, a phenotipic marker of bone resorption, in ligated and non-ligated sites.

MATERIALS AND METHODS

Animals

The study included 36 male Wistar rats, aged 60 days and weighing an average of 194.44 ± 27.52 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 University of Campinas Institutional Care and Use Committee.

Experimental design

The animals were randomly assigned to one of the following experimental groups: G1 (n=12): control healthy animals; G2 (n=12): hypothyroidism, which was induced by the ingestion of a solution with propylthiouracil, an anti-thyroid drug which blocks thyroid hormone synthesis (16) (Propilracil® 100mg, Biolab Sanus Farmacêutica Ltda., Taboão da Serra, SP, Brazil) (1 g / 1 l of drinking water); and G3 (n=12): hyperthyroidism, which was induced by the ingestion of sodium l-thyroxine (T_4) (Puran® T_4 200 µg, Sanofi-Synthelabo Ltda., Rio de Janeiro, RJ, Brazil) and sodium triiodothyronine (T_3) (Sodium Triiodothyronine 90 µg, Drogal Ltda., Piracicaba, SP, Brazil) (800 µg T_4 and 180 µg T_3 / 1 l of drinking water). The animals were continuously administered with the drugs for hormone changes during the whole experimental period, without interruption, as previously reported (17). Figure 1 schematically illustrates the experimental design adopted.

Biochemical serum analyses and ligature placement

Three months after the beginning of the study and, therefore, hormone change induction, blood samples were collected for the assessment of total serum levels of T_3 and T_4 by radioimmunoassay (RIA) (Active Triiodothyronine (T_3) RIA DSL-3100 and Active Thyroxine (T_4) RIA DSL-3200, Diagnostic System Laboratories Inc., Webster, Texas, United States), according to the manufacturer's instructions. This period of dysfunctions induction was maintained to obtain chronic alterations. After the establishment of T_3 and T_4 serum level alterations, one of the mandibular first molars of each animal was randomly assigned to receive a cotton ligature in a submarginal position to induce experimental periodontitis, and the contralateral tooth was left unligated to serve as a control.

Histometric analysis

Thirty days after ligature placement, the animals were sacrificed and the macroscopic characteristics of thyroid glands and periodontal tissues were analyzed. The specimens were then routinely processed and decalcified serial sections (6 μm) were obtained in a mesio-distal direction and stained with hematoxylin and eosin. Using an image analysis system (Image-Pro®, Media Cybernetics, Silver Spring, MD, United States) and a blinded examiner, the area between the bone crest and furcation roof of ligated and non-ligated teeth was histometrically determined by the point counting technique in 10 sections per specimen (18), which were selected every 30 μm. Therefore, the assessed region represented the area of periodontal ligament and periodontal bone loss for non-ligated and ligated teeth, respectively. In addition, five equally distant sections were assessed regarding the bone quality (proportion of mineralized bone tissue in a 1.000-μm zone under the furcation) in the interradicular area of ligated and non-ligated teeth (19). The histometric parameters are schematically illustrated in Figure 2.

TRAP-staining (enzymohistochemistry)

Deparaffinized mesio-distal 6-μm thick sections were incubated at 37 °C for 15 min in a solution prepared by dissolving 4 mg of naphthol AS-BI (Sigma

Chemical Co., St. Louis, MO, United States) and 24 mg of red violet salt (Sigma Chemical Co.) in 30 ml of acetate buffer (pH 5.2) containing 0.3 mmol/l of tartrate (pH 5.0) (Sigma Chemical Co.). Subsequently, sections were washed in distilled water and counterstained with hematoxylin. As a negative control for the TRAP activity, consecutive sections were incubated in substrate-free medium (20). Quantitative analysis of the number of TRAP-positive cells was performed in two different regions described in Figure 2. Firstly, the number of TRAP-positive cells was counted on the linear surface of the bone crest, immediately below the furcation roof in ligated and non-ligated teeth. Secondly, the number of TRAP-positive cells was obtained in the 1000 μm-zone of the interradicular bone under the furcation of the first mandibular molars. The TRAP-positive cells were counted in 2 different sections representative of the mid portion of the tooth. Total area of the interradicular bone and the extension of the alveolar bone surface in the furcation region was obtained using an image analysis system (Image-Pro®, Media Cybernetics, Silver Spring, MD, United States) and the number of TRAP-positive cells, as obtained using a light microscope (Axioskop 2 plus®, Zeiss, Jena, Germany), with a 40x magnification. The results are described as the number of TRAP-positive cells/mm and /mm^2 for the bone crest and the 1000 μm-area under the furcation of the ligated and non-ligated teeth, respectively.

Statistical analysis

Intergroup analyses were carried out by the one-way analysis of variance, ANOVA, to statistically assess quality of tooth-supporting alveolar bone and number of TRAP-positive cells in both ligated and non-ligated sites, the parameters that showed a normal distribution. If statistical differences were detected, a pairwise multiple comparison was additionally performed with the Tukey test. The non-parametric Kruskal-Wallis test was performed for the intergroup comparison when the data did not present a normal distribution, regarding the hormone serum levels and the distance between interradicular bone crest and furcation roof for ligated and non-ligated teeth, respectively. If differences were detected among the groups, the Dunn test was applied. Intragroup analysis was performed using the

paired Student *t* test for all parameters, testing differences between ligated and non-ligated sites. A significance level of 0.05 was adopted for all statistical comparisons.

RESULTS

Clinical observations and biochemical analysis

Macroscopic analysis during autopsies suggested the success of thyroid dysfunctions induction. Thyroid glands in control and hyperthyroidism groups were larger and pink colored, while in hypothyroidism the glands were thin and anemic due to reduced activity. The glands in hyperthyroidism appeared slightly larger than in the control. The total serum levels of T_3 and T_4 before ligature placement confirmed hormone changes and are summarized in Table 1. T_3 and T_4 serum levels were higher in hyperthyroidism ($P = 0.03$ and $P = 0.04$ for T_3 and T_4, respectively) and decreased in hypothyroidism ($P = 0.02$ and $P = 0.03$ for T_3 and T_4, respectively), as compared to control.

In addition, at the time of sacrifice, clinical examination of ligated and non-ligated sites revealed signs of gingival inflammation, including color/volume changes and bleeding in the ligated teeth of all groups; and no signs of inflammation in the non-ligated sites.

Histometric analysis

Histometrically, intragroup analysis showed a significant difference in the distance between interradicular bone crest and furcation roof between non-ligated and ligated teeth in all the experimental groups ($P = 0.0001$, $P = 0.0006$ and $P = 0.0001$ for control, hypothyroidism and hyperthyroidism, respectively). Moreover, an intergroup analysis showed an increased distance between interradicular bone crest and furcation roof, representing a greater bone loss in the ligated teeth for the hypothyroidism group, as compared to the ligated teeth in the control ($P = 0.02$) and hyperthyroidism groups ($P = 0.02$). Furthermore, no statistically significant differences were observed regarding the same area in non-ligated teeth among the

groups (P = 0.08) (Figure 3A). Figures 4A-C illustrate histological findings, showing that there was an increased bone loss in the furcation region of ligated teeth of the hypothyroidism group compared to the control and hyperthyroidism groups.

Regarding the tooth-supporting alveolar bone assessment, data analysis showed, despite the T_3 and T_4 serum levels changes, a similar proportion of mineralized tissue among the experimental groups with the same ligature status (P = 0.24 and P = 0.08, for ligated and non-ligated teeth). On the other hand, an intragroup analysis showed a significant decrease in the proportion of mineralized tissue in the ligated teeth versus the non-ligated teeth in the control (P = 0.02) and hypothyroidism (P = 0.002) groups. Figure 3B graphically illustrates the results described above.

TRAP-staining

The number of TRAP-positive cells was analysed in two different regions. Firstly, for the linear surface of the bone crest, an intergroup analysis showed an increase in the number of TRAP-positive cells/mm in the ligated teeth for the hypothyroidism group, compared to the control (P = 0.01) and hyperthyroidism (P = 0.025) groups. In addition, intragroup analysis demonstrated a significant increase in the number of TRAP-positive cells in ligated teeth than in non-ligated teeth for control (P = 0.01) and hypothyroidism groups (P = 0.001). Figure 3C illustrates the results.

When the number of TRAP-positive cells was evaluated in the 1000μm-zone of the interradicular bone under the furcation of both ligated and non-ligated sites, there was no significant difference among the groups (P = 0.17 and P = 0.91 for ligated and non-ligated teeth, respectively). However, a significant increase in the number of TRAP-positive cells/mm^2 in ligated, compared to non-ligated teeth for each group (P = 0.0001 for control, hypothyroidism and hyperthyroidism), was observed. Figure 3D graphically illustrates the reported data. Figures 4D-F illustrate the TRAP-positive cells in a linear surface on the interradicular bone crest. It is possible to observe that there is an increased number of TRAP-positive cells in the hypothyroid micrograph (4E) as compared to the control and

hyperthyroid micrographs (4D and 4F, respectively). Figures 4G-I show TRAP-positive cells in detail.

DISCUSSION

Thyroid dysfunctions are common health problems in the population and the determination of the influence of thyroid hormones imbalance in periodontitis may be important for the prevention of morbidity related to this condition when the association is present. Thus, the present study aimed at investigating the impact of thyroid hormones on the alveolar bone loss resulting from experimental periodontitis in rats and on the quality of the tooth-supporting alveolar bone. Moreover, the number of TRAP-positive cells on the linear surface of bone crest, immediately below the furcation roof, and in a 1000 μm-area of the interradicular bone under the furcation of the ligated and non-ligated teeth was also assessed. In the present investigation, biochemical analysis demonstrated that the system used was able to induce changes in the serum levels of triiodothyronine (T_3) and thyroxine (T_4), as previously reported (17). Furthermore, data analyses demonstrated that a thyroid hormone deficient state may significantly increase the bone loss resulting from ligature-induced periodontitis, whereas no effect was observed for the opposite condition (increased levels of thyroid hormones). Finally, data analyses further demonstrated that the quality of the tooth-supporting alveolar bone under the furcation was not affected by the thyroid hormone serum levels. Although several studies have reported an association between thyroid hormone levels and skeletal bone mass (9), changes in the microtrabecular alveolar bone following thyroid hormones changes have rarely been reported (21). Thyroid hormones regulate various leucocytic actions such as activation (22) and proliferation of different cellular lineage, including T and B lymphocytes (23). Moreover, thyroid hormones also participate in the release of cytokines such as interferon gamma (INFδ) (24) and interleukin-6 (IL-6) (25). Thus, the decrease in the levels of these hormones may promote a less competent immunogenic

response to the infection induced by the experimental periodontitis (26) and, therefore, it may be suggested that the influence of thyroid hormones on the progression of periodontitis may be related to a relationship between the immune system and the thyroid axis, and not related to the effect of hormone changes on the alveolar bone quality (27). However, further studies should be considered in order to specifically address the effect of thyroid hormones changes in the immune system in the local microenvironment of the periodontium.

Data analysis further demonstrated that the number of TRAP-positive cells may also be affected by lower serum levels of the hypothyroid hormones in sites with periodontitis and, therefore, provide support for the data obtained by histometric analysis in both regions evaluated. TRAP is an iron-containing enzyme whose biological function is not fully known. This enzyme may be involved in the degradation of bone constituents by osteoclasts. Recently, a role for TRAP has been suggested in regulating intracellular vesicular trafficking in resorbing cells (28). High amounts of TRAP are expressed in bone resorbing cells and, therefore, changes in bone resorption are usually associated with changes in the resorbing cells number, suggesting that secreted TRAP may be a useful marker of bone resorption (29). On a molecular basis, in the gingival tissue with periodontal disease, bone resorption is characterized by a primary production of an osteoclast differentiation factor, receptor activator of nuclear factor Kb ligand (RANKL) by B and T cells, which induces differentiation and activation of resorbing cells (30), and may support the increased number of TRAP-positive cells in the ligated versus non-ligated sites in the present study. Moreover, it can be speculated that the more pronounced number of TRAP-positive cells in the linear surface of bone crest and, consequently, more pronounced bone loss in the hypothyroidism group, may also be associated with the upregulation of RANKL in the RANKL/OPG system by altered concentrations of prostaglandins (PGs) and IL-6. These levels of inflammatory molecules are altered as an additional consequence of hypothyroidism or secondary to inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin 1 (IL-1), which regulate osteoclast differentiation

and function independently of the RANKL-RANK interaction (31). However, it remains unclear which factors may trigger the increased differentiation and activity of the resorbing cells in vivo when hypothyroidism is correlated to an infectious process.

In conclusion, within the limits of the present study, in rats, a thyroid hormone-deficient state may potentiate the bone loss resulting from ligature-induced periodontitis, whereas no significant impact of thyroid hormone changes was observed in non-inflamed sites. The findings of the present study suggest that an increased number of TRAP-positive cells in the hypothyroidism condition may play an important role. Therefore, in addition to the importance of thyroid hormone deficiency in the general health status, it may also constitute a critical state with respect to the periodontium, and controlled clinical studies should be considered in order to provide information as to the best approach to deal with this condition.

REFERENCES

1. Kornman KS, Page RC, Tonetti MS. The host response to the microbial challenge in periodontitis: assembling the players. *Periodontol 2000* 1997; **14**: 33-53.

2. Ishikawa I. Host responses in periodontal diseases: a review. *Periodontol 2000* 2007; **43**: 9-13.

3. Grossi SG, Genco RJ, Machtei EE, et al. Assessment of risk for periodontal disease. II. Risk indicators for alveolar bone loss. *J Periodontol* 1995; **66**: 23-29.

4. Genco RJ. Current view of risk factors for periodontal disease. *J Periodontol* 1996; **67** (suppl 10): 1041-1049.

5. Bland R. Steroid hormone receptor expression and action bone. *Clin Sci* 2000; **98**: 217-240.

6. Little JW. Thyroid disorders. Part I: hyperthyroidism. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006; **101**: 276-284.

7. Allain TJ, Thomas MR, McGregor M, Salisbury R. A hystomorphometric study of bone changes in thyroid dysfunction in rats. *Bone* 1995; **16**: 505-509.

8. Vestergaard P, Mosekilde L. Fractures in patients with hyperthyroidism and hypothyroidism: a nationwide follow-up study in 16.249 patients. *Thyroid* 2002; **12**: 411-419.

9. Suwanwalaikorn S, Ongphiphadanakul B, Braverman LE, Baran DT. Differential responses of femoral and vertebral bones to long-term excessive l-thyroxine administration in adults rats. *Eur J Endocrinol* 1996; **134**: 655-659.

10. Siebler T, Robson H, Bromley M, Stevens DA, Shalet SM, Williams GR. Thyroid status affects number and localization of thyroid hormone receptor expressing mast cells in bone marrow. *Bone* 2002; **30**: 259-266.

11. Akalin A, Colak Ö, Alatas Ö, Efe B. Bone remodeling markers and serum cytokines in patients with hyperthyroidism. *Clin Endocrinol* 2002; **57**: 125-129.

12. Siddiqi A, Burrin JM, Wood DF, Monson JP. Tri-iodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. *J Endocrinol* 1998; **157**: 453-461.

13. Tokuda H, Kozawa O, Harada A, Isobe KI, Uematsu T. Triiodothyronine modulates interleukin-6 synthesis in osteoblasts: inhibitions in protein kinase A and C pathways. *Endocrinology* 1998; **139**: 1300-1305.

14. Bergström J, Eliasson S, Dock J. A 10-year prospective study of tobacco smoking and periodontal health. *J Periodontol* 2000; **71**: 1338-1347.

15. Taylor GW. Bidirectional interrelationships between diabetes and periodontal diseases: an epidemiologic perspective. *Ann Periodontol* 2001; **6**: 99-112.

16. Abraham P, Avenell A, Park CM, Watson WA, Bevan JS. A systematic review of drug therapy for Grave's hyperthyroidism. *Eur J Endocrinol* 2005; **153**: 489-498.

17. Feitosa DS, Bezerra BB, Ambrosano GMB, et al. Thyroid hormones may influence cortical bone healing around titanium implants. A histometric study in rats. *J Periodontol* 2008; **79**: 881-887.

18. Duarte PM, Assis DR, Casati MZ, Sallum AW, Sallum EA, Nociti FH Jr. Alendronate may protect against increased periodontitis-related bone loss in estrogen-deficient rats. *J Periodontol* 2004; **75**: 1196-1202.

19. César-Neto JB, Benatti BB, Sallum EA, Casati MZ, Nociti-Júnior FH. The influence of cigarette smoke inhalation and its cessation on the tooth-supporting alveolar bone: a histometric study in rats. *J Periodontol Res* 2006; **41**: 118-123.

20. Cole AA, Walters LM. Tartrate-resistant acid phosphatase in bone and cartilage following decalcification and col embedding in plastic. *J Histochem Cytochem* 1987; **35**: 203-206.

21. Talaeipour AR, Shirazi M, Kheirandish Y, Delrobaie A, Jafari F, Dehpour AR. Densitometric evaluation of skull and jaw bones after administration of thyroid hormones in rats. *Dentomaxillofac Radiol* 2005; **34**: 332-336.

22. Botella-Carretero JI, Prados A, Manzano L, et al. The effects of thyroid hormones on circulating markers of cell mediated immune response, as studied in patients with differentiated thyroid carcinoma before and during thyroxine withdrawal. *Eur J Endocrinol* 2005; **153**: 223-230.

23. Klecha AJ, Genaro AM, Gorelik G, et al. Integrative study of hypothalamus-pituitary-thyroid-immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. *J Endocrinol* 2006; **189**: 45-55.

24. Lin HY, Martino LJ, Wilcox BD, Davies FB, Gordinier JK, Davies PJ. Potentiation by thyroid hormone of human IFN-gamma-induced HLA-DR expression. *J Immunol* 1998; **161**: 843-9.

25. Kim C-H, Kim H-K, Shong Y K, Lee K-U, Kim GS. 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. *J Endocrinol* 1999; **160**: 97-102.

26. Bucay AH. Clinical hypothesis: application of AIDS vaccines together with thyroid hormones to increase their immunogenic effect. *Vaccine* 2007; **25**: 6292-6293.

27. Klecha AJ, Genaro AM, Lysionek AE, Caro RA, Colluccia AG, Cremaschi GA. Experimental evidence pointing to the bidirectional interaction between the immune system and the thyroid axis. *Int J Immunopharmacol* 2000; **22**: 491-500.

28. Perez-Amodio S, Jansen DC, Schoenmaker T, et al. Calvarial osteoclasts express a higher level of tartrate-resistant acid phosphatase than long bone osteoclasts and activation does not depend on cathepsin K or L activity. *Calcif Tissue Int* 2006; **79**: 245-254.

29. Halleen JM, Tiitinen SL, Ylipahkala H, Fagerlund KM, Väänänen HK. Tartrate-resistant acid phosphatase 5b (TRACP 5b) as a marker of bone resorption. *Clin Lab* 2006; **52**: 499-509.

30. Han X, Kawai T, Taubman MA. Interference with immune-cell-mediated bone resorption in periodontal disease. *Periodontol 2000* 2007; **45**: 76-94.

31. Kanatani M, Sugimoto T, Sowa H, Kobayashi T, Kanzawa M, Chihara K. Thyroid hormone stimulates osteoclast differentiation by a mechanism independent of RANKL-RANK interaction. *J Cell Physiol* 2004; **201**: 17-25.

ACKNOWLEDGMENTS

Dr. Feitosa gratefully thanks the The National Council for Scientific and Technological Development – CNPq, Brasília, DF, Brazil, for their financial support.

TABLE

Table 1. Mean and standard errors of serum levels of triiodothyronine (ng/dl) and thyroxine (µg/dl) achieved in each experimental group.

Group	Triiodothyronine (ng/dl)	Thyroxine (µg/dl)
Control	111.53 ± 5.03	2.64 ± 0.17
Hypothyroidism	78.07 ± 3.50 *	1.28 ± 0.05 *
Hyperthyroidism	182.07 ± 9.06 *	4.18 ± 0.25 *

* represents statistically significant difference between test (hypothyroidism and hyperthyroidism) and control groups ($P < 0.05$) by the Kruskal-Wallis test and Dunn test.

FIGURES

Figure 1.

Illustration of the experimental design.

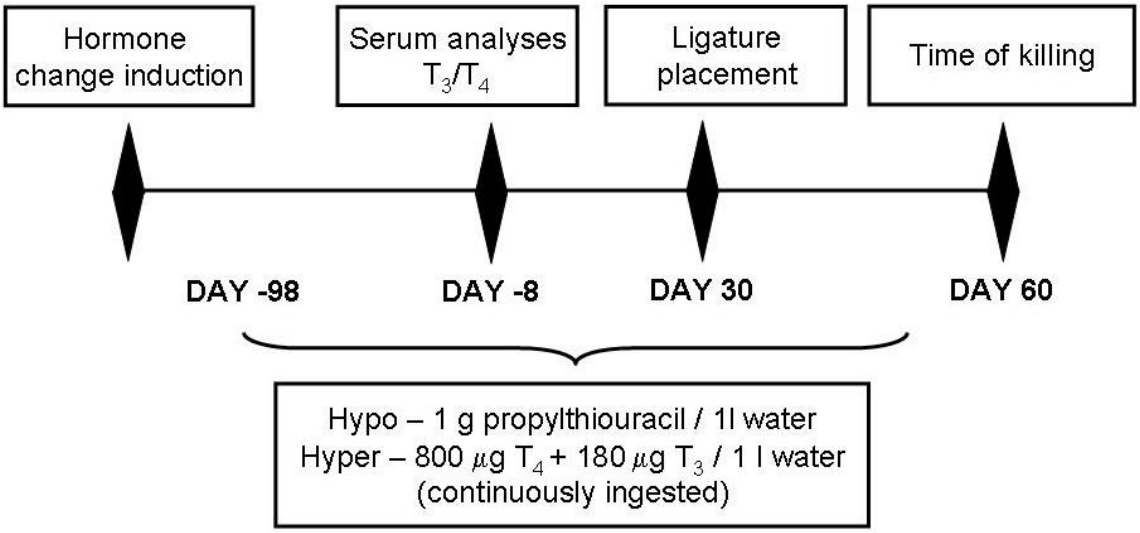
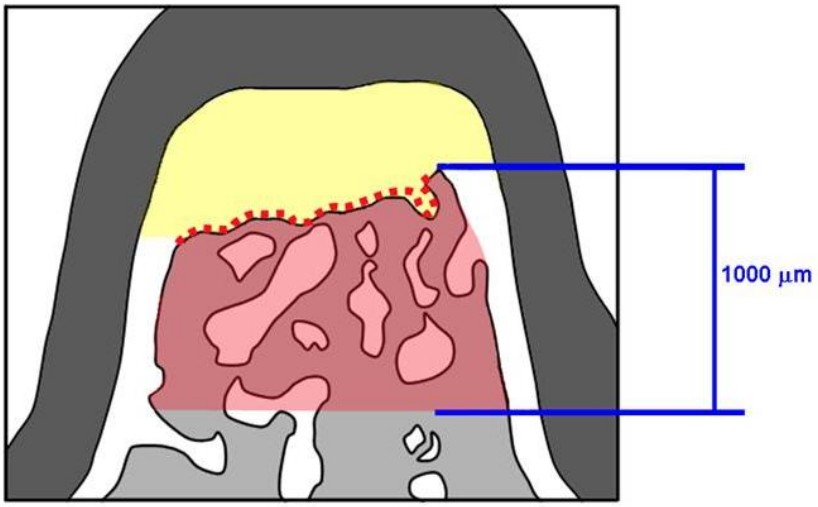


Figure 2. Schematic illustration of the regions for histometric analysis, including bone loss/periodontal ligament areas and bone quality; and for TRAP-positive cells counted on the linear surface of the bone crest and in a 1000-μm zone under the furcation.



Area between bone crest and cementum surface equivalent to the area of bone loss or periodontal ligament in ligated and non-ligated teeth, respectively.

Area of mineralized bone tissue in a 1000-μm zone under the furcation where bone quality and the number of TRAP-positive cells/mm^2 were analysed .

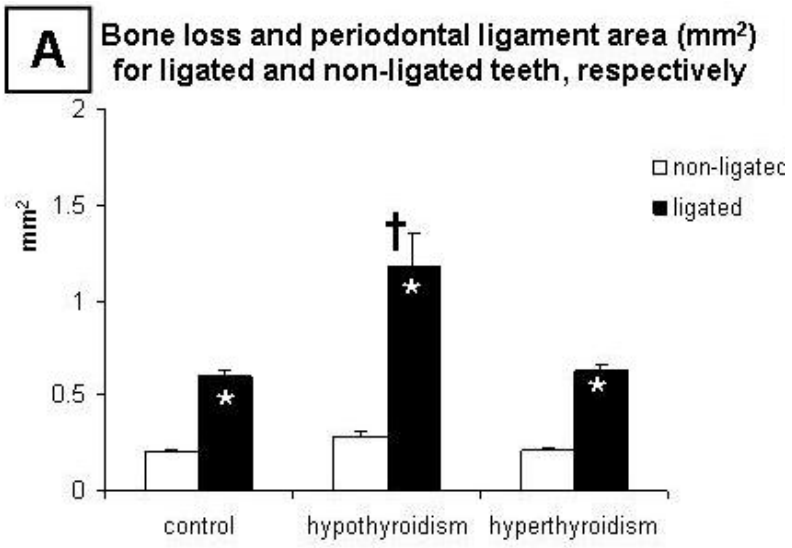
Linear surface of bone crest below the furcation roof where the number of TRAP-positive cells/mm was analysed.

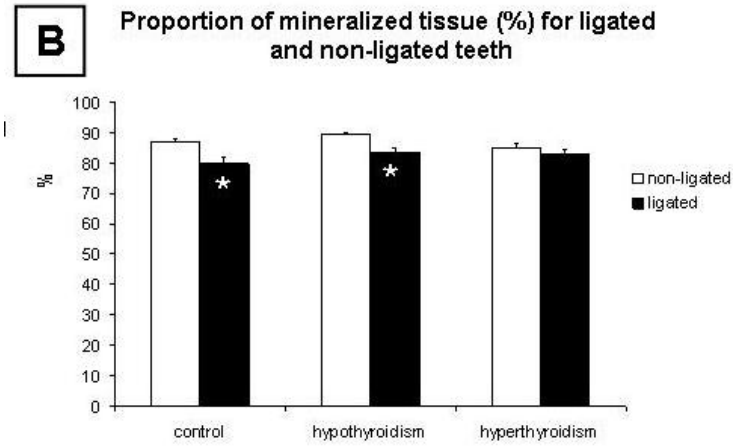
Figure 3. The illustration graphically represents the means and the standard errors of the study parameters achieved in ligated and non-ligated sites for control, hypothyroidism and hyperthyroidism groups. A. Bone loss and periodontal ligament area for ligated and non-ligated teeth, respectively (mm^2). B. Proportion of mineralized tissue (%). C. Number of TRAP-positive cells/mm in a linear surface on the bone crest. D. Number of TRAP-positive cells/mm^2 in a 1000-μm zone under the furcation.

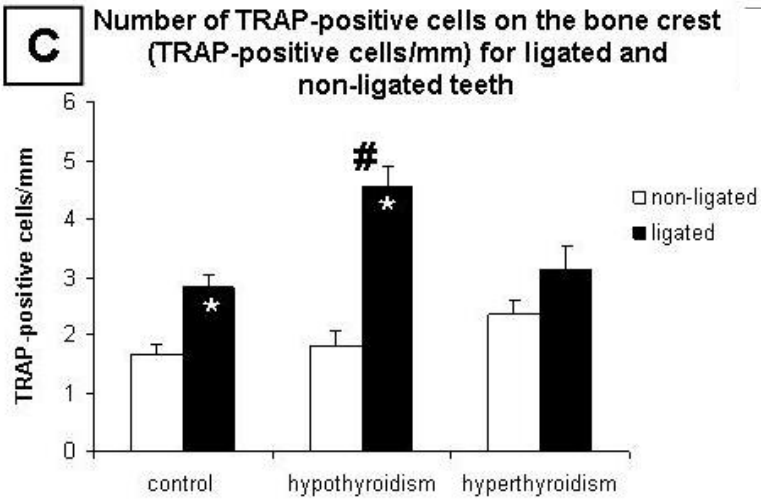
* Statistically different by an intra-group comparison (ligated versus non-ligated teeth) (P ≤ 0.05), by the paired Student *t*-test.

† Statistically different by an intergroup comparison (control versus hypothyroidism versus hyperthyroidism) (P ≤ 0.05), by the Kruskal Wallis test followed by Dunn test.

Statistically different by an intergroup comparison (control versus hypothyroidism versus hyperthyroidism) (P ≤ 0.05), by one-way analysis of variance (ANOVA) followed by the Tukey test.




B
Proportion of mineralized tissue (%) for ligated and non-ligated teeth
%
100
90
80
70
60
50
40
30
20
10
0
control
hypothyroidism
hyperthyroidism
non-ligated
ligated


C
Number of TRAP-positive cells on the bone crest (TRAP-positive cells/mm) for ligated and non-ligated teeth
TRAP-positive cells/mm
6
5
4
3
2
1
0
#
control
hypothyroidism
hyperthyroidism
non-ligated
ligated

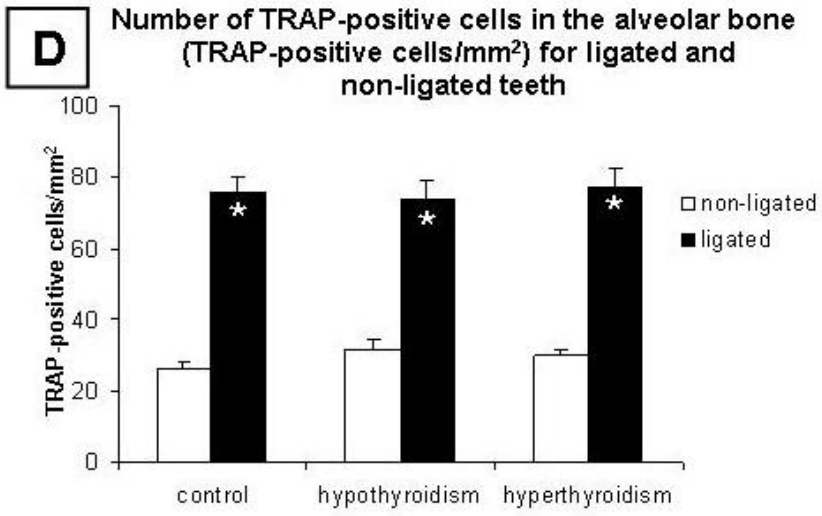
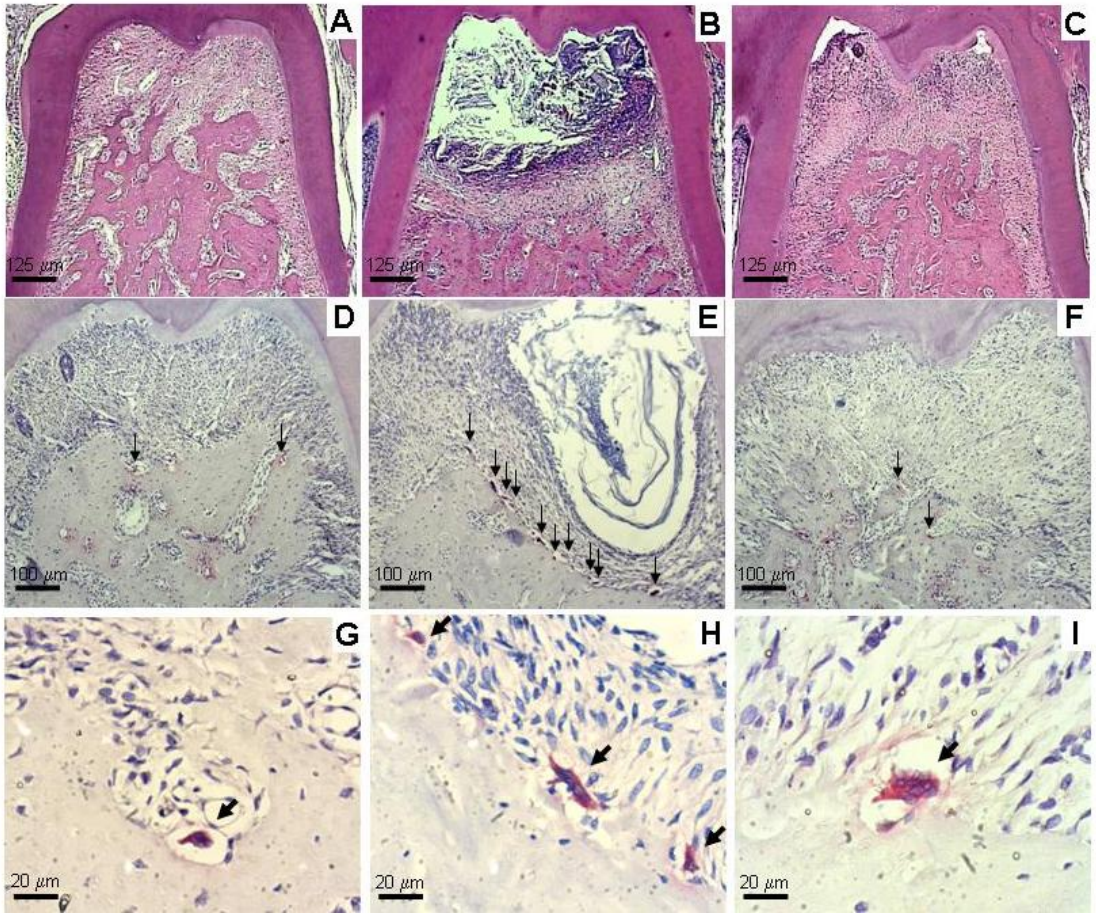

D
Number of TRAP-positive cells in the alveolar bone (TRAP-positive cells/mm²) for ligated and non-ligated teeth
TRAP-positive cells/mm²
100
80
60
40
20
0
control
hypothyroidism
hyperthyroidism
non-ligated
ligated

Figure 4. Photomicrographs illustrating the histologic aspects of bone loss in the furcation region and TRAP-positive cells on the linear surface of the bone crest immediately below the furcation roof in the ligated teeth for control (A, D), hypothyroidism (B, E) and hyperthyroidism groups (C, F) (hematoxilin and eosin/TRAP counterstaining with hematoxilin; original magnification 5x/10x; bar=125 μm/100 μm, respectively for histometric/TRAP analysis). Photomicrographs G-I show amplified details of TRAP-positive cells (TRAP counterstaining with hematoxilin; original magnification 50x; bar=20 μm).



CONCLUSÃO

Dentro dos limites do presente estudo é possível concluir que:

- Os hormônios tireoidianos parecem capazes de influenciar a densidade mineral e a proporção de osso cortical na tíbia de ratos, mas a mandíbula parece ser um osso menos sensível a variações nos níveis destes hormônios.
- A deficiência dos hormônios tireoidianos pode ser capaz de aumentar a progressão da perda óssea induzida pela periodontite em ratos, em função de um aumento do número de células com capacidade de reabsorção óssea.

REFERÊNCIAS*

Abu EO, Bord S, Horner A, Chatterjee VKK, Compston JE. The expression of thyroid hormone receptors in human bone. Bone. 1997; 21(2): 137-42.

Akalin A, Colak Ö, Alatas Ö, Efe B. Bone remodeling markers and serum cytokines in patients with hyperthyroidism. Clin Endocrinol. 2002; 57: 125-29.

Allain TJ, Thomas MR, McGregor M, Salisbury R. A histomorphometric study of bone changes in rats. Bone. 1995; 16(5): 505-9.

Bergström J, Eliasson S, Dock J. A 10-year prospective study of tobacco smoking and periodontal health. J Periodontol. 2000; 71: 1338-47.

Bjoro T, Holmen J, Krüger O, Midthjell K, Hunstad K, Schreiner T *et al*. Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large unselected population. The health study of Nord-Trondelag (HUNT). Eur J Endocrinol. 2000; 143: 639-47.

Bland R. Steroid hormone receptor expression and action bone. Clin Sci. 2000; 98: 217-40.

Britto JM, Fenton AJ, Holloway WR, Nicholson GC. Osteoblasts mediate thyroid hormone stimulation of osteoclastic bone resorption. Endocrinology. 1994; 134(1): 169-76.

Campus G, Salem A, Uzzau S, Baldoni E, Tonolo G. Diabetes and periodontal disease: a case-control study. J Periodontol. 2005; 76(3): 418-25.

Devlin H, Horner K. Diagnosis of osteoporosis in oral health care. J Oral Reabil. 2008; 35: 152-57.

Eriksen EF, Mosekilde L. Trabecular bone remodeling and bone balance in hyperthyroidism. Bone. 1985; 6: 421-28.

Guyton AC, Hall JE. Os hormônios metabólicos da tireóide. In: Guyton AC, Hall JE. Tratado de fisiologia médica. 10 ed. Rio de Janeiro: Guanabara Koogan; 2002. p. 802-12.

* De acordo com a norma da UNICAMP/FOP, baseadas na norma do International Commitee of Medical journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

Jacobs R, Ghyselen J, Koninckx P, van Steenberghe D. Long-term bone mass evaluation of mandible and lumbar spine in a group of women receiving hormone replacement therapy. Eur J Oral Sci. 1996; 104: 10-16.

Jaffin RA, Berman CL. The excessive loss of Branemark fixtures in type IV bone: A 5-year analysis. J Periodontol. 1991; 62(1): 2-4.

Jeffcoat M. The association between osteoporosis and oral bone loss. J Periodontol. 2005; 76(11suppl): 2125-32.

Jodar E, Munoz-Torres M, Escobar-Jimenez F, Quesada M, Luna JD, Olea N. Antiresorptive therapy in hyperthyroid patients: longitudinal changes in bone and mineral metabolism. J Clin Endocrinol Metab. 1997; 82: 1989-94.

Kinane DF, Hart TC. Genes and gene polymorphisms associated with periodontal disease. Crit Rev Oral Biol Med. 2003: 14(6): 430-49.

Kornman KS. Mapping the pathogenesis of periodontitis: a new look. J Periodontol. 2008; 79: 1560-68.

Little JW. Thyroid disorders. Part I: hyperthyroidism. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(3): 276-84.

Milne M, Kang MI, Quail JM, Baran DT. Thyroid hormone excess increases insulin-like growth factor I transcripts in bone marrow cell cultures: divergent effects on vertebral and femoral cell cultures. Endocrinology. 1998; 139(5): 2527-34.

Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y *et al*. A novel interaction between thyroid hormones and $1,25(OH)_2D_3$ in osteoclast formation. Biochem Biophys Res Commun. 2002; 291: 987-94.

Numbenjapon N, Costin G, Gilsanz V, Pitukcheewanont P. Low cortical bone density measured by computed tomography in children and adolescents with untreated hyperthyroidism. J Pediatr. 2007; 150: 527-30.

Oetting A, Yen PM. New insight into thyroid hormone action. Best Pract Res Clin Endocrinol Metab. 2007; 21(2): 193-208.

Oishi K, Ishida H, Nishikawa S, Hamasaki A, Wakano Y. The effect of triiodothyronine on cell growth and alkaline phosphatase activity of isolated rat calvarial cells. J Bone Miner Res. 1990; 5: 229.

Ongphiphadhanakul B, Alex S, Braverman LE, Baran DT. Excessive l-thyroxine therapy decreases femoral bone mineral densities in the male rat: effect of hypogonadism and calcitonin. J Bone Miner Res. 1992; 7:1227-31.

Ongphiphadhanakul B, Jenis LG, Braverman LE, Alex S, Stein GS, Lian JB *et al*. Eridronate inhibits the thyroid hormone-induced bone loss in rats assessed by bone mineral density and messenger ribonucleic acid markers of osteoblast and osteoclast function. Endocrinology. 1993; 133(6): 2502-7.

Peruzzo DC, Benatti BB, Antunes IB, Andersen ML, Sallum EA, Casati MZ *et al*. A systematic review of stress and psychological factors as possible risk factors for periodontal diseases. J Periodontol. 2007; 79(4): 697-704.

Pontes AAN, Adan LF, Costa ADM, Benício AVL, Silva CRA, Morais RM *et al*. Prevalência de doenças da tireóide em uma comunidade do nordeste brasileiro. Arq Bras Endocrinol Metabol. 2002; 46(5): 544-9.

Rizzoli R, Poser J, Bürgi U. Nuclear thyroid hormone receptors in cultured bone cells. Metabolism. 1986; 35(1): 71-74.

Schimid C, Schläpfer I, Futo E, Waldvogel M, Schwander J, Zapf J, Froesch ER. Triiodothyronine (T3) stimulates insulin-like growth factor (IGF)-1 and IGF binding protein (IGFBP)-2 production by rat osteoblasts in vitro. Acta Endocrinol (Copenh) 1992; 126(5): 467-73.

Siddiqi A, Burrin M, Noonan K, James I, Wood DF, Price CP *et al*. A longitudinal study of markers of bone turnover in Graves' disease and their value in predicting bone mineral density. J Clin Endocrinol Metab. 1997; 82: 753-9.

Siddiqi A, Burrin JM, Wood DF, Monson JP. Tri-iodothyronine regulates the production of interleukin-6 and interleukin-8 in human bone marrow stromal and osteoblast-like cells. J Endocrinol. 1998; 157: 453-61.

Silverthorn DU, Ober WC, Garrison CW, Silverthorn AC. Equilíbrio energético, metabolismo e crescimento. In: Silverthorn DU, Ober WC, Garrison CW, Silverthorn AC. Fisiologia Humana. Uma abordagem integrada. 1 ed. Rio de Janeiro: Editora Manole; 2003. p. 667-80.

Suwanwalaikorn S, Ongphiphadhanakul B, Braverman LE, Baran DT. Differential responses of femoral and vertebral bones to long-term excessive l-thyroxine administration in adults rats. Eur J Endocrinol. 1996; 134(5): 655-9.

Talaeipour AR, Shirazi M, Kheirandish Y, Delrobaie A, Jafari F, Dehpour AR. Densitometric evaluation of skull and jaw bones after administration of thyroid hormones in rats. Dentomaxillofac Radiol. 2005; 34: 332-36.

Vestergaard P, Mosekilde L. Fractures in patients with hyperthyroidism and hypothyroidism: a nationwide follow-up study in 16,249 patients. Thyroid. 2002; 12(5): 411-19.

Wactawski-Wende J, Hausmann E, Hovey K, Trevizan M, Grossi S, Genco RJ. The association between osteoporosis and alveolar crestal height in postmenopausal women. J Periodontol. 2005; 76: 2116-24.

Williams GR, Bland R, Sheppard MC. Characterization of thyroid hormone (T3) receptors in three osteosarcoma cell lines of distinct osteoblastic phenotype: interactions among T3, vitamin D, and retinoid signaling. Endocrinology. 1994; 135: 2375-85.

UNICAMP

Universidade Estadual de Campinas
Instituto de Biologia

CEEA-IB-UNICAMP

Comissão de Ética na Experimentação Animal
CEEA-IB-UNICAMP

C E R T I F I C A D O

Certificamos que o Protocolo nº **1003-1**, sobre **"INFLUÊNCIA DE ALTERAÇÕES TIREOIDIANAS NA PROGRESSÃO DA DOENÇA PERIODONTAL E NO REPARO ÓSSEO AO REDOR DE IMPLANTES DE TITÂNIO INSERIDOS EM TÍBIAS. ESTUDO HISTOMÉTRICO EM RATOS"** sob a responsabilidade de **Prof. Dr. Sérgio de Toledo / Daniela da Silva Feitosa** está de acordo com os Princípios Éticos na Experimentação Animal adotados pelo Colégio Brasileiro de Experimentação Animal (COBEA), tendo sido aprovado pela Comissão de Ética na Experimentação Animal (CEEA)-IB-UNICAMP em reunião de 17 de abril de 2006.

C E R T I F I C A T E

We certify that the protocol nº **1003-1**, entitled **"INFLUENCE OF THYROID DISEASES IN THE PROGRESSION OF PERIODONTITIS AND IN THE BONE REPAIR AROUND TITANIUM IMPLANTS PLACED IN TIBIAE. HISTOMETRIC STUDY IN RATS"**, is in agreement with the Ethical Principles for Animal Research established by the Brazilian College for Animal Experimentation (COBEA). This project was approved by the institutional Committee for Ethics in Animal Research (State University of Campinas - UNICAMP) on April 17, 2006.

Campinas, 17 de abril de 2006.

Profa. Dra. Ana Maria A. Guaraldo
Presidente

Fátima Alonso
Secretária Executiva

CEEA/IB – Unicamp
Caixa Postal 6109
13083-970 Campinas, SP – Brasil

Telefone: (19) 3788-6359
Telefax: (19) 3788-6356
E-mail: ceea@cemib.unicamp.br
http://www.ib.unicamp.br/institucional/ceea/index.htm

J Periodont Res 2009; 44: 472–478

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2008.01144.x

The influence of thyroid hormones on periodontitis-related bone loss and tooth-supporting alveolar bone: a histological study in rats

D. S. Feitosa[1], M. R. Marques[2], M. Z. Casati[1], E. A. Sallum[1], F. H. Nociti Jr[1], S. de Toledo[1]
Departments of [1]Prosthodontics and Periodontics, Division of Periodontics and [2]Morphology, Division of Histology, School of Dentistry at Piracicaba, State University of Campinas, Piracicaba, Brazil

Feitosa DS, Marques MR, Casati MZ, Sallum EA, Nociti Jr FH, de Toledo S. The influence of thyroid hormones on periodontitis-related bone loss and tooth-supporting alveolar bone: a histological study in rats. J Periodont Res 2009; 44: 472–478.

Background and Objective: Recent studies have pointed to potentially periodontal risk indicators, however no information is available on the impact of changes in thyroid hormone levels on the progression of periodontitis and on the quality of alveolar bone. Thus, the aim of the present study was to evaluate histologically, in rats, the influence of thyroid hormones on the rate of periodontal bone loss resulting from ligature placement and on the quality of tooth-supporting alveolar bone.

Material and Methods: Thirty-six male Wistar rats were randomly assigned to the following groups: healthy (control, $n = 12$), hypothyroidism ($n = 12$) and hyperthyroidism ($n = 12$). Once alterations were confirmed by total serum levels of triiodothyronine and thyroxine, ligatures were randomly placed around one of the first mandibular molars. Thirty days later, the animals were killed and specimens routinely processed for serial decalcified sections. The parameters assessed were periodontitis-related bone loss, quality of tooth-supporting alveolar bone and the number of cells positive for tartrate-resistant acid phosphatase (TRAP), a marker of bone resorption.

Results: At the ligated sites, intergroup analysis revealed that hypothyroidism significantly increased the bone loss resulting from ligature-induced periodontitis ($p = 0.02$) and the number of TRAP-positive cells on the linear surface of bone crest ($p = 0.01$). In addition, no significant differences were detected regarding the quality of the bone ($p = 0.24$) or the number of TRAP-positive cells in the area of the interradicular bone for ligated teeth among the groups ($p = 0.17$).

Conclusion: It may be concluded that decreased serum levels of thyroid hormones may enhance periodontitis-related bone loss, as a function of an increased number of resorbing cells, whereas the tooth-supporting alveolar bone seems to be less sensitive to alterations in hormone levels.

Daniela da Silva Feitosa, DDS, MS, School of Dentistry at Piracicaba, University of Campinas, Avenida Limeira 901, Areião, 13414-903, Piracicaba, São Paulo, Brazil
Tel: +55 19 2106 5301
Fax: +55 19 2106 5301
e-mail: dsfeitosa@yahoo.com or danielafeitosa@fop.unicamp.br

Key words: periodontitis; alveolar bone; triiodothyronine; thyroxine

Accepted for publication June 3, 2008