RICARDO AYELLO GUERRA

A TIROGLOBULINA É AINDA DETECTÁVEL NO SORO DE INDIVÍDUOS NORMAIS APÓS 3 MESES DE USO DE L-TIROXINA EM DOSE SUPRESSIVA DE TSH

Tese apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina para a obtenção do título de Mestre em Ciências da Saúde

SÃO PAULO
2006

RICARDO AYELLO GUERRA

A TIROGLOBULINA É AINDA DETECTÁVEL NO SORO DE INDIVÍDUOS NORMAIS APÓS 3 MESES DE USO DE L-TIROXINA EM DOSE SUPRESSIVA DE TSH

Tese apresentada à Universidade Federal de São Paulo – Escola Paulista de Medicina para a obtenção do título de Mestre em Ciências da Saúde

Orientador: Prof. Dr. Rui M. B. Maciel

Coordenador do Programa de Pós-Graduação em Endocrinologia: Prof. Dr. Sérgio A. Dib

SÃO PAULO

2006

Dedico este trabalho aos meus pais: Yolanda e Lupércio, ao meu irmão Guilherme, à minha esposa querida e a Leonardo, meu filhote..

AGRADECIMENTOS

Agradeço ao meu orientador, doutor Rui Monteiro de Barros Maciel. Sua amizade, companheirismo e confiança foram as chaves para a conclusão desta tese. Esteve sempre ao meu lado, disponível e solicito. Soube respeitar minhas dificuldades e utilizar minhas capacidades para construir esta pequena obra que hoje apresentamos. Só posso agradecê-lo por ter acreditado em mim e me dado a oportunidade de, sob sua inestimável orientação, tentar. Muito obrigado doutor Rui.

Agradeço ao doutor Reinaldo Furlanetto por sua amizade e confiança. Seu bom senso, aliado a todo o seu conhecimento médico são o que o tornam um clínico e professor tão especial. Também à doutora Luiza K. Matsumura por tudo o que me ensinou no trato das patologias da tiróide. Por fim, à doutora Elza Ikejiri que, sempre prestativa, realizou com grande perícia e paciência as ultra-sonografias de nosso trabalho.

Agradeço ao meu bom amigo Evandro Portes que, ao iniciar seus trabalhos no Hospital do Servidor Público Estadual, foi meu grande incentivador, ao lado da minha esposa Luciana, para iniciar este projeto que hoje concluímos. Agradeço à minha grande amiga Cláudia Nakabashi, que esteve sempre ao meu lado, trocando experiências, dividindo angústias, mas com um otimismo e tranqüilidade contagiantes, que foram fundamentais para chegar ao fim deste processo.

Agradeço aos meus colegas de Escola Paulista que tanto me ajudaram e aconselharam, especialmente Rosa Paula e Cléber, Bel, Dani, Cláudia Akemi, Ana Cristina e João Roberto, além de todos os outros que contribuíram para o ambiente agradável em que permaneci neste tempo.

Agradeço aos amigos que fiz no laboratório de biologia molecular, Gustavo, Gisele, Rosana, Janete Cerutti, e especialmente Teresa Kasamatsu e Felipe Crispim, por toda a ajuda que deram para as dosagens hormonais efetuadas. Em especial também à Ângela, amiga, fraterna e sempre pronta a ajudar.

Agradeço à Amaryllis e a todos os funcionários da Disciplina de Endocrinologia que tornaram possível este trabalho, em especial à Ivonete, que

tanto me ajudou na coleta e estocagem do material, sem sua ajuda, este trabalho jamais sairia.

Agradeço às minhas chefias dos dois Hospitais em que trabalho por disponibilizarem, dentro do possível, tempo para que eu pudesse realizar esta tese. Em especial ao doutor Bernardo Lichewitz, do HSPE, pela confiança recém depositada em mim.

Agradeço aos meus preceptores de residênca, doutores Mozart Novaes Jr, Nazaré Bromberg, Rubens Werner, Chady Farah e ao doutor João H. Romaldini por todo o ensinamento que me deram.

Agradeço a todos os meus colegas dos locais onde trabalho, Lenira, Cláudio Capuano, Ana Beatriz, Márcio Vendramini, Maria da Conceição Mamone e tantos outros bons amigos que venho colecionando e que me dão o prazer de sua companhia.

Agradeço a Alex e Marcela Perazo, José Estevão e Patrícia Lima, Otávio Andrade, Maria do Socorro, José Luis e Fabiana, meus grandes amigos, por me fazerem consciente do valor que tem uma amizade verdadeira. Incluo na lista acima Guilherme, que além de melhor amigo, é também irmão. Sua presença foi, é e sempre será fundamental para o meu sucesso.

Agradeço aos meus pais queridos pelo exemplo que sempre me foram e pelo amor incondicional que sempre me tiveram. Agradeço a minha esposa amada que, além de grande incentivadora, é hoje parte de mim, proporcionando-me, a cada dia, os momentos mais felizes de minha vida. Agradeço também aos meus sogros, meu bom amigo seu Carlos e à dona Eugènia, e a toda família de minha esposa e a todos de minha extensa família, em especial à minha prima e sempre grande amiga Patrícia.

Agradeço ao Laboratório Fleury por ter disponibilizado a estrutura para realizar as dosagem de anticorpos anti-tiroperoxidase. Também ao laboratório Merck por fornecer os comprimidos de L-tiroxina (Euthyrox ®) necessários.

Por fim, agradeço de todo o coração ao que chamarei de O Residente Amigo. Há quase nove anos exerço a função de preceptor nos dois Hospitais em que trabalho, o Hospital do Servidor Público Estadual e o Hospital do Servidor

Público Municipal. O estudo ao lado dos residentes destes Hospitais foi o grande incentivo para que eu entrasse nesta empreitada que hoje concluímos. Não citarei o nome de nenhum, pois seria injusto incluir uns e não citar todos. Moram todos em meu coração, são a verdadeira razão deste trabalho, além de amigos que ganho ano após ano. Espero ter a oportunidade de aplicar tudo o que aprendi nesta Escola com e para vocês. Muito obrigado.

ÍNDICE

1. APRESENTAÇÃO

1. APRESENTAÇÃO

Nesta tese de mestrado, apresentamos, de acordo com recomendações do Conselho Especial do Programa de Pós-Graduação de Endocrinologia da Universidade Federal de São Paulo – Escola Paulista de Medicina, um trabalho completo em inglês entitulado: "Thyroglobulin is still detectable in the serum of normal individuals after a trial of TSH-suppressive doses of L-T4 during 3 months", traduzido para o português como "A tiroglobulina é ainda detectável no soro de indivíduos normais após 3 meses de uso de L-tiroxina em dose supressiva de TSH", de autoria de Ricardo A. Guerra, Felipe Crispim, Cláudia C. D. Nakabashi, Cléber P. Camacho, Teresa S. Kasamatsu e Rui M. B. Maciel.

A idéia deste trabalho surgiu a partir da necessidade de conhecimento do comportamento dos níveis séricos de tiroglobulina na ausência de um dos três principais fatores determinantes de sua concentração, os níveis séricos de TSH (Spencer e cols., 1996; Torréns e Burch, 2001; Baloch e cols., 2003). Além do interesse fisiológico, há também uma questão prática envolvida, que é o diagnóstico precoce da tirotoxicose factícia (Mariotti e cols., 1982; Bogazzi e cols., 1999).

2. INTRODUÇÃO

INTRODUÇÃO

A tiróide é uma glândula localizada na região cervical antero-inferior. Em sua anatomia, é constituída por dois lobos, medindo cada um cerca de 4,0 cm de altura x 2,0 cm de largura x 2,0 cm de profundidade, unidos por um fino istmo de tecido tiroidiano, que mede 2,0 x 2,0 x 0,5 cm (Larsen e cols., 2003). Histologicamente, a glândula é composta por uma infinidade de folículos, sua unidade funcional. Um folículo é uma estrutura esférica constituído por uma única camada de células foliculares (ou tirócitos) que envolve um interior de material proteináceo denominado colóide, composto basicamente pela proteína tiroglobulina (Tg) (Larsen e cols., 2003).

A célula folicular tem como função produzir hormônios tiroidianos. Para tanto, ela capta iodo do meio extra-celular através de seu simportador de sódio-iodo (conhecido como NIS, do inglês "sodium-iodide symporter") (Eskandari e cols., 1997; De La Vieja e cols., 2000). Do interior da célula folicular, este iodo é transportado para o colóide onde, sob a ação da enzima tiroperoxidase (TPO) é oxidado e acoplado a um radical tirosil presente em uma molécula de Tg. Estes resíduos de iodotirosinas sofrem processo de acoplamento, também catalisado pela TPO, causando a formação de resíduos de iodotironinas (Ohmyia e cols., 1990; Taurog e cols., 1996; Carrasco, 2005).

Para catalisar as reações descritas acima, a enzima TPO necessita da presença de peróxido de hidrogênio (H_2O_2). A geração deste cofator se dá no interior do tirócito, através da ação de uma flavoproteína que oxida NADPH em um sistema Ca^{2+} dependente. Dois cDNAs capazes de codificar oxidases tiroidianas de NADPH foram clonados. Estas oxidases são normalmente designadas THOX1 e THOX2 (do inglês "thyroid oxidase"), mas suas designações oficiais são DUOX1 e DUOX2 (referente a "dual oxidase") (Carrasco, 2005).

Em uma segunda etapa, uma molécula de Tg é captada por mecanismo de pinocitose do colóide, transportada para o interior da célula em uma vesícula que se fundirá com um lisossomo. Nesta organela, catepsinas promoverão a hidrólise da molécula de Tg, com a conseqüente liberação das iodotironinas, triiodotironina

(T3) e tetraiodotironina ou tiroxina (T4), que são os hormônios que a glândula tiróide secreta (Bernier-Valentin e cols., 1990; Kostrouch, 1991; Arvan e di Jeso, 2005).

Síntese da Tg

A tiroglobulina é uma glicoproteína traduzida a partir de um gene extremamente extenso localizado no cromossomo 8q24,2-q24,3 (Baas e cols., 1985). A transcrição deste gene e de outros, importantes para o funcionamento normal do tirócito, como a tiroperoxidase (TPO) e o simportador sódio-iodo (NIS), é estimulada pela tirotrofina hipofisária (TSH), seja de forma direta, seja indiretamente, ao induzir a síntese de fatores de transcrição como *TTF1*, *FOXE1* e *PAX-8* (Van Herle e cols., 1979; Ohno e cols., 1999; De Felice e Di Lauro, 2004).

Após a transcrição, o mRNA da Tg abandona o núcleo e é traduzido por um complexo polirribossômico. A proteína monomérica resultante possui peso molecular de 330 kDa, é composta por 2.748 aa e apresenta coeficiente de sedimentação de 12S. Esta penetra no retículo endoplasmático, onde sofrerá glicosilação em até 30 pontos distintos de sua estrutura. A proteína é transferida para o complexo de Golgi, onde conclui o processo de glicosilação e, finalmente, é envolta numa vesícula (em cuja parede há a enzima TPO) que a secretará para o colóide. Nesta vesícula secretora inicia-se o processo de fusão de dois monômeros idênticos para formar um homodímero de 660 kDa (Arvan e di Jeso, 2005).

Uma vez no colóide, sob influência da enzima TPO, a Tg sofre iodação. Cada dímero de 660 kDa possui um total de 134 resíduos tirosil, estando 30 destes disponíveis para a ligação com o iodo. A ligação de um resíduo de tirosil com um átomo de iodo gera uma monoiodotirosina (MIT) e a dois átomos de iodo, uma diiodotirosina (DIT). Em geral, uma molécula de Tg possui cerca de 26 átomos de iodo em sua estrutura final. O próximo passo será o acoplamento, quando há a "cessão" do conteúdo do anel iodado de uma iodotirosina para outra, com a conseqüente formação de resíduos com 3 iodos, tri-iodotironina, T3

(produto de DIT + MIT) ou 4 iodos, tetra-iodotironina ou tiroxina (produto de DIT + DIT), conhecidos como iodotironinas (Bernier-Valentin e cols., 1990; Kostrouch, 1991; Arvan e di Jeso, 2005).

A molécula "madura" de Tg é uma glicoproteína homodimérica com peso molecular de 660 kDa e coeficiente de sedimentação de 19S (Arvan e di Jeso, 2005). A Tg "madura" ficará estocada no colóide até que seja recolhida por uma vesícula de pinocitose para hidrólise.

Como se explica a presença de moléculas intactas de Tg na circulação sanguínea periférica?

Apesar da Tg, via de regra, ser submetida à hidrólise para produção de hormônios tiroidianos, algumas destas moléculas escapam deste processo e podem ser encontradas intactas na circulação sanguínea (Hjort, 1961)

Moléculas de Tg com diferentes conteúdos de iodo estão presentes no colóide. Estudos recentes sugerem que as moléculas de Tg com menor conteúdo de iodo apresentam uma facilidade estrutural de se ligar a receptores presentes na membrana apical do tirócito denominados megalina (Druetta e cols., 1999; Lisi e cols., 2003).

A megalina é uma proteína com um único domínio transmembrana que, ao se ligar à molécula de Tg, promove sua transcitose, ou seja, permite que a Tg atravesse toda a célula sem que a mesma sofra hidrólise e a secreta intacta pela membrana basolateral (Marinò e McCluskey, 2000; Marinò e cols., 2000; Marinò e cols., 2001).

Conseqüentemente, existe um mecanismo intrafolicular capaz de separar a Tg "pobre" em iodo, secretando-a intacta, sem que se gaste a maquinaria enzimática de lise com uma proteína capaz de gerar pouco ou nenhum hormônio (Druetta e cols., 1999; Lisi e cols., 2003). Ainda nesta linha, recente estudo demonstra que ratos “knock-out” para o gene da megalina (Meg $^{-/-}$) exibem hipotiroidismo, devido à competição existente entre moléculas de Tg "ricas" e "pobres" em iodo pelas vesículas de pinocitose (Lisi e cols., 2005).

Fatores que influenciam os níveis séricos de Tg

Há três fatores que influenciam diretamente a concentração sanguínea da Tg: os níveis séricos de TSH, a massa de tecido tiroidiano existente e a injúria à glândula tiróide (Van Herle e cols., 1973; Spencer e Wang, 1995; Torréns e Burch, 2001; Baloch e cols., 2003).

Dosagem dos níveis séricos de Tg – técnicas e utilidade clínica

A mensuração dos níveis séricos de Tg pode ser feita a partir de sangue periférico. Atualmente, esta mensuração é normalmente realizada por meio de método imunométrico, que possui boa sensibilidade e especificidade (Baloch e cols., 2003). Resultados falsos-negativos podem ocorrer na presença de anticorpos anti-tiroglobulina no soro utilizado; desta forma, recomenda-se sempre a dosagem dos níveis séricos destes anticorpos concomitantemente à determinação da Tg (Preisner e cols., 2003; Spencer, 2004; Spencer e cols., 2005).

A mensuração dos níveis séricos de Tg possui uma utilidade clínica fundamental, que é a de servir para o acompanhamento do carcinoma diferenciado de tiróide após a tiroidectomia total ou quase-total e após a ablação de restos tiroidianos com radioiodo. Nestas circunstâncias, uma vez que o tirócito é a única fonte de Tg do organismo, a detecção de Tg sinalizará a presença de restos tiroidianos que não sofreram ablação ou a presença de metástases (Mazzaferri e Kloos, 2001; Mazzaferri, 1999; Schlumberger e cols., 2004).

Outra utilidade da dosagem dos níveis séricos de Tg é no diagnóstico diferencial da tirotoxicose. Quando esta é causada por hiperfuncionamento da glândula (hipertiroidismo), seja de causa primária ou secundária, os níveis séricos de Tg encontram-se claramente elevados. Por outro lado, pacientes com tirotoxicose devido ao uso inadvertido de altas doses de hormônio tiroidiano (tirotoxicose factícia) apresentam níveis séricos indetectáveis desta proteína (Torréns e Burch, 2001; Mariotti e cols., 1982; Bogazzi e cols., 1999); entretanto,

os estudos demonstrativos destes resultados realizaram-se com pacientes que vinham usando doses extremamente elevadas de hormônios tiroidianos há um período de tempo desconhecido, possivelmente longo (Mariotti e cols., 1982; Bogazzi e cols., 1999).

Até o momento, não há estudos prospectivos que demonstrem o real comportamento da Tg logo após o início do uso de doses supra-fisiológicas de L-tiroxina. Um estudo como este nos pareceu interessante, pois contribuiria tanto para um melhor entendimento da fisiologia da Tg, quanto para abreviar o diagnóstico de tirotoxicose factícia, problema comum em nosso país, mas que pode tornar-se também frequente em outras regiões, onde a adição dos hormônios tiroidianos a medicamentos ditos emagrecedores está acontecendo (Ohye e cols., 2005).

Sendo assim, o objetivo deste estudo é analisar o comportamento dos níveis séricos de Tg em indivíduos eutiroidianos durante o uso de doses supressivas de L-tiroxina por um período de três meses.

3. Thyroglobulin is still detectable in the serum of normal individuals after a trial of TSH-suppressive doses of L-T4 during 3 months

Thyroglobulin is still detectable in the serum of normal individuals after a trial of TSH-suppressive doses of L-T4 during 3 months

Ricardo A. Guerra, Felipe Crispim, Claudia C. D. Nakabashi, Cleber P. Camacho, Teresa S. Kasamatsu and Rui M. B. Maciel

Division of Endocrinology and Metabolism, Department of Medicine, Escola Paulista de Medicina, Federal University of São Paulo, São Paulo, Brazil

Correspondence:

Rui M. B. Maciel, M.D., Ph.D.
Laboratory of Molecular Endocrinology
Division of Endocrinology, Department of Medicine
Escola Paulista de Medicina, Universidade Federal de São Paulo
Rua Pedro de Toledo, 781, 12º floor
São Paulo, SP 04039-032
Brazil
Phone/fax: +55-11-5084-5231
e-mail: rmbmaciel@pesquisa.epm.br

ABSTRACT

Objective: Three factors can influence the production of thyroglobulin (Tg) and its serum concentrations, serum levels of TSH, thyroid mass and glandular injury. In this study we have evaluated serum levels of thyroglobulin in normal individuals without the influence of TSH.

Materials and methods: 19 healthy euthyroid individuals (12 F/7 M, age range: 21-36 years) were enrolled. In the first visit, history, physical examination and thyroid ultrasound were performed and blood sample was collected. Inclusion criteria were negative serum anti-thyroid antibodies, normal serum TSH and normal thyroid ultrasound. Exclusion criteria were smoking, previous thyroid disease or any contraindication for suppressive L-T4 therapy. The volunteers received L-T4 (mean dose: 2.09 ± 0.12 μg/kg/day) and were seen each 14 days for examination and blood collection (total of 7 visits).

Results: As expected, there was a decay in serum TSH levels (mean basal = 1.70 ± 0.79 mU/L; 14d = 0.10 ± 0.10 mU/L; 28d = 0.02 ± 0.05 mU/L; and remained suppressed thereafter) and an increase in FT4. Thyroglobulin levels, however, did not decreased as TSH; its levels had a decrease only in the first 14 days but recovered to values very similar to the basal and remained stable until the end of the use of LT4 (mean basal = 3.97 ± 2.50 ng/mL; 14 d = 2.15 ±1.70 ng/mL; 28d = 3.24 ± 3.28 ng/mL, remaining at similar, detectable values thereafter).

Conclusion: Our findings suggest that serum levels of thyroglobulin do not shift significantly in healthy individuals who use LT4 for a period of 84 days. There are two main mechanisms for thyroglobulin uptake in thyroid colloid, pynocitosis and megalin-induced. The suppression of TSH probably turned-off both of them. Despite this, the amount of megalin already present at the apical cell membrane could still bind thyroglobulin and transcytose it into the blood. By this hypothesis, this is a time-limited process and, if these volunteers had used L-T4 for a longer time, probably all the pre-formed megalin would be used and thyroglobulin levels would be undetectable (as found in factitious thyrotoxicosis).

INTRODUCTION

Thyroglobulin (Tg), the precursor of thyroid hormones, is a 660-kDa glycoprotein synthesized by thyrocytes and secreted into the follicle lumen, where it is stored as the major component of colloid (1,2). Hormone release requires retrieval of Tg from the colloid by thyrocytes and proteolytic cleavage along the lysosomal pathway (1,2). However, some internalized Tg bypasses the lysosomal pathway and it is secreted intact into the bloodstream (3,4).

Tg is secreted exclusively by thyrocytes and, consequently, can be measured as a specific marker of the presence of these cells in the body. This allows the measurement of Tg serum levels to check the presence of metastatic tissues in patients treated for differentiated thyroid carcinoma after total thyroidectomy and radioiodine ablation (2,5,6). This can be done during thyroid hormone suppressive therapy or, as a more sensitive test, with elevated TSH levels either after thyroid hormone withdrawal or recombinant human TSH injection (2,7,8).

Although the main utility of the measurement of serum Tg is the follow-up of thyroid carcinoma, there are other clinical conditions where it can be important, one of them being the differentiation between endogenous and exogenous thyrotoxicosis (2,9-14). Whatever its use, it is crucial to interpret serum Tg with the concomitant observation of the 3 factors that can influence its levels, i.e.: (1) the thyroid mass; (2) the presence of thyroid injury, and (3) the TSH concentration (2,11,12).

There is a general agreement that 1 g of normal thyroid tissue results in a serum Tg of approximately 1 ng/mL when TSH is in the normal range and about 0.5 ng/mL when TSH is suppressed (2). Studies in patients with factitious thyrotoxicosis suggests that chronic exposure to low TSH levels leads to a decrease of serum Tg to undetectable levels (9,13). However, there are no prospective trials analyzing the acute effect of TSH suppression in euthyroid healthy individuals.

In this trial, our objective is to evaluate the behavior of serum Tg in normal subjects while excluding the influence of TSH on its values.

MATERIALS AND METHODS

We have enrolled 19 healthy euthyroid individuals (12 females and 7 males, age range: 21-36 year-old). In the first visit a history was taken, a physical examination and a thyroid ultrasound were performed and blood sample was collected. The inclusion criteria included negative levels of serum antithyroid antibodies (anti-Tg and anti-peroxidase antibodies), normal serum values of TSH and a normal thyroid on ultrasonography [normal thyroid size (mean volume: 8.45 mL; range: 5.2-12.8 mL) and morphology]. Patients with a history of smoking or previous thyroid disease were excluded, as well as those who were pregnant or had psychiatric or cardiovascular disease or any other contraindication for the use of suppressive L-T4 therapy.

The volunteers received L-T4 in a dose of approximately 2.0 μg/kg/day (mean L-T4 dose was 2.09 ± 0,12 μg/kg/day). That dose would be adjusted after 2 months to obtain a serum TSH less than 0.1 mU/L, but, by this time, everyone had a suppressed TSH. Volunteers were seen at 0, 14, 28, 42, 56, 70 and 84 days. At each visit, they were examined for signs and symptoms of thyrotoxicosis and blood was collected.

The blood was collected into a tube without anticoagulant, the samples were allowed to clot for at least 15 minutes and then centrifuged at 4,000 rpm for 10 minutes. The serum was removed and stored at -20º C and the samples of each individual were measured in the same assay.

The protocol was approved by the Ethics Committee of the Hospital São Paulo, the main teaching hospital of the University and all patients gave written informed consent.

Methods

Serum TSH was measured by an in-house immunofluorimetric assay (15), free T4 (FT4) by a time-resolved fluoroimmunoassay (DELFIA, PerkinElmer Life

and Analytical Sciences, Wallak Oy, Turku, Finland), anti-TPO antibodies by RIA (Brahms, Berlin, Germany), anti-thyroglobulin antibodies by an in-house immunofluorimetric assay (16) and Tg by a time-resolved fluoroimmunoassay (DELFIA, PerkinElmer Life and Analytical Sciences, Wallak Oy, Turku, Finland). All serum measurements were done in duplicate. Between-assay coefficient of variation were calculated as 8% for TSH, 3.5% for FT4 and 5,5% for Tg. The imprecision profile for TSH at serum concentrations of 0.05-100 mU/L was 8%; for FT4 at serum concentrations of 0.2-5.8 ng/dL was 11%; for Tg at serum concentrations of 1.0-1000 ng/mL was 8%; and for anti-Tg antibodies at serum concentrations of 15.6-1000 U/mL was 5,1%. Normal values in our population are 0.5-5.0 mU/L for TSH; 0.5-1.6 ng/dL for FT4 and 1-30 ng/mL for Tg. Anti-TPO antibodies were considered negative when less than 60 U/mL and anti-Tg antibodies when less than 40 U/mL. The sensitivity of the assays are: TSH: 0.05 mU/L; FT4: 0.2 ng/dL and Tg: 1.0 ng/mL. When TSH or Tg values were lower than these, a 0 value was attributed.

Ultrasound imaging was performed with an ALOKA SSD-500 instrument using a linear transducer of 7,5 MHz. All tests were done by the same experienced operator at the time of the enrollment of the volunteer to the protocol. The operator had no access to the data of patients. The total thyroid volume was measured as previously described (17).

Statistical analysis

Tg, TSH and FT4 data are presented as mean and standard deviation in each evaluation time. We have also constructed the mean profile (mean + standard error) to analyze the time effect. Time effect was evaluated by a variance analysis (ANOVA) with repeated measures. This has permitted the statistical modeling of the structural correlations between data. PROC MIXED from SAS version 9.1 was used for statistical analysis of the data. The level of statistical significance was as p-value <0,05.

RESULTS

We tolerated a maximum of 2 absences in the scheduled visits. Between the 19 participants who fulfilled this criteria, 12 (63.2%) had no absence, 6 (11.6%) had 1 absence and 1 (5.2%) had 2 absences. Analyzing time by time, we had 0 absences in time 0, 1 absence in times 1, 2, 4 and 5 and 2 absences in times 3 and 6, respectively. In fact, 25 individuals have entered the protocol, but 1 was discharged because he had more 2 absences in the scheduled visits and 5 stopped their participation for variable reasons: one because of personal reasons, one because of dizziness, two because detection of mild irregular heart beats (extra-systoles) and one because of migraine associated with the start of L-T4. Of the symptoms attributed to L-T4, the participant who related dizziness did not had alleviation after 45 d off L-T4. Migraine and irregular heart beats had disappeared after L-T4 withdrawal.

At 14 days of L-T4 use, mean serum levels of TSH were already 0.1 mU/L (0.1 ± 0.1 mU/L) (Table 1). At 28 days these levels were clearly suppressed (0.02 ± 0.05 mU/L) and remained so thereafter (Figure 1). Maintained suppressed levels of TSH from 28 days until the end in all the 19 volunteers reflect the compliance with the protocol. Another relevant data is that the mean heart rate of the participants suffered an increase from a baseline value of 83 ± 10 beats per minute (bpm) to a peak at 42 days (93 ± 11 bpm – 11,7% above the basal) but thereafter we were able to see a progressive decrease in this parameter, reaching 86 ± 13 bpm – 3,6% above the basal – at the end of the study (84 days of L-T4 use).

Mean serum levels of FT4 had an inverse behavior: at 14 days of L-T4 use, they were already above the normal (1.85 ± 0.48 ng/dL) and remained so thereafter (Table 1).

Thyroglobulin levels, however, did not decreased, as TSH. Its levels had a decrease only in the first 14 days, but recovered to values similar to the basal and remained stable until the end of the use of LT4 (Figure 2). The values obtained at baseline were not statistically different from that at the last time (mean basal =

3.97 ± 2.50 ng/mL x 84 days = 4.65 ± 3.38 ng/mL ($p = 0.101$) (Figure 1) (Table 2).

DISCUSSION

This trial has shown that when euthyroid individuals are subjected to TSH suppression, Tg cannot be suppressed and were still detected in most of them during all 3 months of the protocol.

Tg basal levels were very near of the lower limit of its normal range. Although the number of individuals analyzed is not sufficient to suggest a lowering of normal values, the results are in agreement with the finding from other authors (18,19), who recognize that in healthy subjects living in geographical areas with normal iodine intake, serum Tg levels are usually low or undetectable.

The choice of the dose of L-thyroxine was based on the fact that the recommended replacement dose for patients with hypothyroidism is 1.6 to 1.7 μg/kg/day (20-22). The dose of L-thyroxine used (~2.0 μg/kg/day) was able to suppress TSH levels in all participants. Most of them exhibited some minor side effects (increase in heart rate and mild shortness of sleep-time were the most common), but tolerated it well, as noted by the low rate of abandon.

Mean serum levels of Tg described a subtle decay in the first 14 days of L-T4 use, but at 28 days returned to levels very similar to those seen at the baseline, and thus remained until the end of the protocol. These levels were detectable at all times. Another authors analyzing thyroglobulin levels in patients with suppressed TSH due to surreptitious ingestion of high doses of L-T4 (thyrotoxicosis factitia) have often found them undetectable (9,13). Thus, Mariotti et al. (9) have studied Tg levels in 6 patients with factitious thyrotoxicosis and have found them below the detectable limit (< 1.25 ng/mL) in all cases. These patients were using elevated doses of thyroid hormones for a long period of time and this is probably the reason for the difference with our results. Bogazzi et al (13) described a series of 25 women in which, even using elevated doses of L-thyroxine, probably by 12-18 months, Tg was in low detectable levels (9-11 μg/L – sensitivity < 3 μg/L) in 3 of them.

The follicle, the thyroid functional unit, is composed of a single layer of epithelial cells (thyrocytes) surrounding a lumen containing colloid (1). Tg is the major protein component of the colloid, into which it is secreted after synthesis by the thyrocytes, a mechanism controlled by TSH. There is evidence that Tg secretion is a regulated, rather than a constitutive process (23,24). This may be useful in achieving balance between release and uptake which, under normal conditions, must be equal (4).

Within the colloid, much of the Tg is insoluble and, therefore, not readily available for uptake, but the concentration of soluble Tg available for thyrocytes is probably also relatively high (4). Consequently, the process of internalization and degradation of Tg by thyroid cells must be strictly regulated to provide appropriate amounts of thyroid hormone and to avoid excessive hormone release. Several mechanisms – receptor-mediated or not – participate of the process of Tg internalization. The most important are fluid-phase nonspecific micropinocytosis, by which high Tg amounts are transported to lysosomes, where they are degraded with release of T3 and T4 into the circulation, and megalin-mediated, by which it is transported by transcytosis from the apical to the basolateral cell surface, where it is released intact by exocytosis into the bloodstream (4).

Megalin was first identified by Kerjaschki and Farquhar (25) and later found to be a member of the low-density-lipoprotein receptor family. It is composed of a single-transmembrane domain, a large ectodomain and a short cytoplasmic tail (26-28). Megalin mediates tubular uptake of low-molecular-weight proteins in the kidney and is also involved in the development of the central nervous system (29). At the thyroid, megalin is expressed in a TSH-dependent manner, and located on the apical surface of the thyrocytes (30). As noted above, megalin is responsible for the Tg transcytosis, a process that serves to divert Tg molecules with a low hormone content from lysosomes, thereby favoring lysosomal degradation of hormone-rich Tg molecules resulting in a more effective hormone release (31,32).

Based on the above mentioned, we speculate that in our study, when TSH became suppressed, it turned-off the mechanism of colloid pynocitosis, as well as

megalin gene expression. Despite this, thyroglobulin could still be found in the serum of the participants. We think that, although the production of new megalin was blocked, the megalin molecules already present at the apical cell membrane could still bind thyroglobulin and transcytose it into the blood. By this hypothesis, this is a time-limited process and if the volunteers have used L-T4 for a longer period, probably all the megalin would be used and thyroglobulin levels would fall and became undetectable (as found in factitious thyrotoxicosis).

Several years ago, at the end of an editorial on thyrotoxicosis factitia, Hamolsky (33) raised the question of how long it would be "...before our ingenious Munchausen learns to obtain and self administer some thyroglobulin..." to confound us by eliminating the clue diagnostic feature (low/undetectable serum Tg) of thyrotoxicosis factitia. This has not occurred yet but our data warrants clinicians to do not discharging the diagnosis of factitious thyrotoxicosis if a detectable Tg is obtained. This may happen, at least in individuals using suppressive, but not very high L-thyroxine doses in a less than 3 months period. The importance of this is emphasized when we note that the age of patients using elevated doses of L-thyroxine in a surreptitious way seems to be increasing (13) and even mild thyrotoxicosis may be associated with adverse health outcomes. Patients with subclinical thyrotoxicosis, particularly the elderly, have a 3- to 6-fold increased risk for atrial fibrilation (34,35). Women with subclinical thyrotoxicosis, particularly those who are estrogen-deficient suffer an increased rate of loss of bone mineral and fractures (36,37). Subclinical thyrotoxicosis in the elderly has also been associated with increased risk of all cause, as well as cardiovascular mortality (38).

Finally, a recent report describes the addition of thyroid hormones to "herbal pills" used as weight-reducers (39). If this turns a current practice, the importance of the above data discussed can be greatly magnified.

In conclusion, our findings suggest that Tg serum levels do not shift significantly in healthy individuals who use LT4-suppressive doses for a period of 84 days.

REFERENCES

1. Arvan P, di Jeso B (2005) Thyroglobulin structure, function, and biosynthesis. in Werner and Ingbar´s The Thyroid Fundamental and Clinical Text, eds Braverman LE & Utiger RD (Lippincott, Williams & Wilkins, Philadelphia), pp 77-95

2. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF et al. 2003 Guidelines Comittee National Academy of Clinical Biochemistry. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 13: 3-126

3. Hatipoglu BA, Schneider AB 1999 Selective endocytosis of thyroglobulin; a review of potential mechanisms for protecting newly synthesized molecules from premature degradation. Biochimie 81: 549-555

4. Marinò M, McCluskey RT 2000 Role of thyroglobulin endocytic pathways in control of thyroid hormone release. Am J Physiol Cell Physiol 279: C1295-1306

5. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ et al. 2006 Management guidelines for patients with thyroid nodules and differentiated thyroid cancer-The American Thyroid Association Guidelines Task Force Thyroid 16: 1-34

6. Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti JS 2005 Clinical impact of thyroglobulin (Tg) and Tg-autoantibody method differences on the management of patients with differentiated thyroid carcinoma J Clin Endocrinol Metab 90: 5566-5575

7. Mazzaferri EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L et al. 2003 A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 88: 1433-1441

8. Schlumberger M, Pacini F, Wiersinga WM, Toft A, Smit JW, Sanchez-Franco F et al. 2004 Follow-up and management of differentiated thyroid carcinoma: an European perspective in clinical practice. Eur J Endocrinol 151: 539-548

9. Mariotti S, Martino E, Cupini C, Lari R, Giani C, Baschieri L and Pinchera A 1982 Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia. N Engl J Med 307: 410-412

10. Cohen JH, Ingbar SH and Braverman LE 1989 Thyrotoxicosis due to ingestion of excess thyroid hormone. Endocr Rev 10: 113-124

11. Spencer CA, Wang CC 1995 Thyroglobulin measurement, techniques, clinical benefits, and pitfalls. Endocrinol Metab Clin N Am 24: 841-863

12. Spencer CA, Takeuchi M, Kazarosyan M 1996 Current status and performance goals for serum thyroglobulin assays. Clin Chem 42: 164-173

13. Bogazzi F, Bartalena L, Scarcello G, Campomori A, Rossi G and Martino E 1999 The age of patients with thyrotoxicosis factitia in Italy from 1973 to 1996. J Endocrinol Invest 22: 128-133

14. Torréns J and Burch HB 2001 Serum thyroglobulin measurement. Endocrinol Metab Clin N Am 30: 429-67

15. Vieira JGH, Kunii IS, Nishida SK, Matsumura LK, Russo EMK, Maciel RMB 1992 Development and application of a immunofluorimetric method for the measurement of human TSH in serum and in total blood coolected in filter paper. Arq Bras Endocrinol Metab 36: 7-12

16. Vieira JGH, Tachibana TT, Fonseca RMG, Nishida SK, Maciel RMB 1996 Development of a immunofluoroassay for the measurement of thyroglobulin antibodies. Arq Bras Endocrinol Metab 40: 232-237

17. Hegedüs, L 1990 Thyroid size determined by ultrasound. Influence of physiological factors and non-thyroidal disease. Dan Med Bull 37: 249-263

18. Buchinger W, Lorenz-Wawschineck O, Semlitsch G, Langsteger W, Binter G, Bonelli RM, Eber O 1997 Thyrotropin and thyroglobulin as an index of optimal iodine intake: correlation with iodine excretion of 39,913 euthyroid patients. Thyroid 7: 593-597

19. van den Briel T, West CE, Hautvast JGAJ, Vulsma T, Vijlder JJM, Ategbo EA 2001 Serum thyroglobulin and urinary iodine concentration are the most

appropriate indicators of iodine status and thyroid function under conditions of increasing iodine supply in schoolchildren in Benin. J Nutr 131: 2701-2706

20. Fish LH, Schwartz HL, Cavanaugh J, Steffes MW, Bantle JP, Oppenheimer JH 1987 Replacement dose, metabolism and bioavailability of levothyroxine in the treatment of hypothyroidism. N Engl J Med 316: 764-770

21. Toft AD 1994 Thyroxine therapy. N Engl J Med 331: 174-180

22. Roberts CGP, Ladenson PW 2004 Hypothyroidism. Lancet 363: 793-803

23. Kim PS, Arvan P 1993 Hormonal regulation of thyroglobulin export from the endoplasmic reticulum of cultured thyrocytes. J Biol Chem 268: 4873-4879

24. Arvan P, Kim PS, Kuliawat R, Prabakaran D, Muresan Z, Yoo SE, Abu Hossain S 1997 Intracellular protein transport to the thyrocyte plasma membrane: potential implications for thyroid physiology. Thyroid 7: 89-105

25. Kerjaschki D, Farquhar MG 1982 The pathogenic antigen of Heymann nephritis is a membrane glycoprotein of the renal proximal tubule brush border. Proc Natl Acad Sci USA 79: 5557-5581

26. Raychowdhury R, Niles JL, McCluskey RT, Smith JA 1989 Autoimmune target in Heymann nephritis is a glycoprotein with homology to the LDL receptor. Science 244: 1163-1165

27. Saito A, Pietromonaco S, Loo AKC, Farquhar MG 1994 Complete cloning and sequencing of rat gp330 "megalin", a distinctive member of the low density lipoprotein receptor gene family. Proc Natl Acad Sci USA 91: 9725-9729

28. Hjalm GE, Murray G, Crumley W, Harazim S, Lundgren I, Onyango B et al. 1996 Cloning and sequencing of human gp330, a Ca2+-binding receptor with potential intracellular signaling properties. Eur J Biochem 239: 132-137

29. Christensen EI, Birn H 2002 Megalin and cubilin: multifunctional endocytic receptors. Nat Rev Mol Cell Biol 3: 256-266

30. Marinò M, Zheng G, Chiovato L, Pinchera A, Brown D, Andrews D and McCluskey RT 2000 Role of megalin (gp330) in transcytosis of thyroglobulin by thyroid cells. A novel function in the control of thyroid hormone release. J Biol Chem 275: 7125-7138

31. Lisi S, Pinchera A, McCluskey RT, Willnow TE, Refetoff S, Marcocci C et al. 2003 Preferential megalin-mediated transcytosis of low-hormonogenic thyroglobulin: a control mechanism for thyroid hormone release. Proc Natl Acad Sci USA 100: 14858-14863

32. Lisi S, Segnani C, Mattii L, Botta R, Marcocci C, Dolfi A et al. 2005 Thyroid dusfunction in megalin deficient mice. Mol Cel Endocrinol 236: 43-47

33. Hamolsky MW Truth is stranger than factitious 1982 N Engl J Med 307: 436-7

34. Sawin CT, Geller A, Wolf PA, Bleanger AJ, Baber E, Bacharach P, Wilson PW, Benjamin EJ and D´Agostino RB 1994 Low serum thyrotropin concentrations as a risk factor for atrial fibrilation in older persons. N Engl J Med 331: 1249-1252

35. Auer J, Scheiber P, Mische T, Langsteger W, Eber O and Eber B 2001 Subclinical hyperthyroidism as a risk factor for atrial fibrilation. Am Heart J 142: 838-842

36. Bauer DC, Ettinger B, Nevitt M and Stone KI 2001 The Study of Osteoporotic Fractures Research Group. Risk for fracture in women with low serum levels of thyroid-stimulating hormone. Ann Intern Med 134: 561-568

37. Vestergaard P, Mosekilde L 2002 Fractures in patients with hypothyroidism and hypothyroidism: a nationwide follow-up study in 16,249 patients. Thyroid 12: 411-419

38. Parle JV, Maisonneuve P, Sheppard MC, Boyle P and Franklyn JA 2001 Prediction of all-cause and cardiovascular mortality in elderly people from one low serum thyrotropin result: a 10-year cohort study. Lancet 358: 861-865

39. Ohye H, Fukata S, Kanoh M, Kubota S, Kuma K, Miyauchi A, Sugawara M 2005 Thyrotoxicosis caused by weight-reducing herbal medicines. Arch Intern Med 165: 831-834

Table 1. Tg, TSH and FT4 (mean ± sd) in all times of the protocol

Time of L-T4 use	**Tg** (ng/mL)	**TSH** (mU/L)	**FT4** (ng/dL)
Basal	3,97 ± 2,50 (n=19)	1,70 ± 0,79 (n=19)	1,06 ± 0,14 (n=19)
14 days	2,15 ± 1,70 (n=18)	0,10 ± 0,10 (n=18)	1,85 ± 0,48 (n=18)
28 days	3,24 ± 3,28 (n=18)	0,02 ± 0,05 (n=18)	2,10 ± 0,57 (n=18)
42 days	4,80 ± 3,92 (n=17)	0,01 ± 0,03 (n=16)	2,01 ± 0,46 (n=16)
56 days	4,86 ± 3,50 (n=18)	0,00 ± 0,01 (n=18)	2,07 ± 0,52 (n=18)
70 days	5,06 ± 4,40 (n=18)	0,00 ± 0,00 (n=18)	2,08 ± 0,65 (n=18)
84 days	4,65 ± 3,38 (n= 18)	0,02 ± 0,06 (n=18)	1,94 ± 0,47 (n=18)

Table 2. Comparison between Tg serum concentration results of the different times

	14days	**28 days**	**42 days**	**56 days**	**70 days**	**84 days**
0	p<0,001	p=0,286	p=0,551	p=0,161	p=0,100	p=0,101
14 days	-	p=0,072	p=0,010	p<0,001	p<0,001	p<0,001
28 days	-	-	p=0,018	p=0,010	0,007	p=0,004
42 days	-	-	-	p=0,431	p=0,232	p=0,277
56 days	-	-	-	-	p=0,428	p=0,688
70 days	-	-	-	-	-	p=0,427

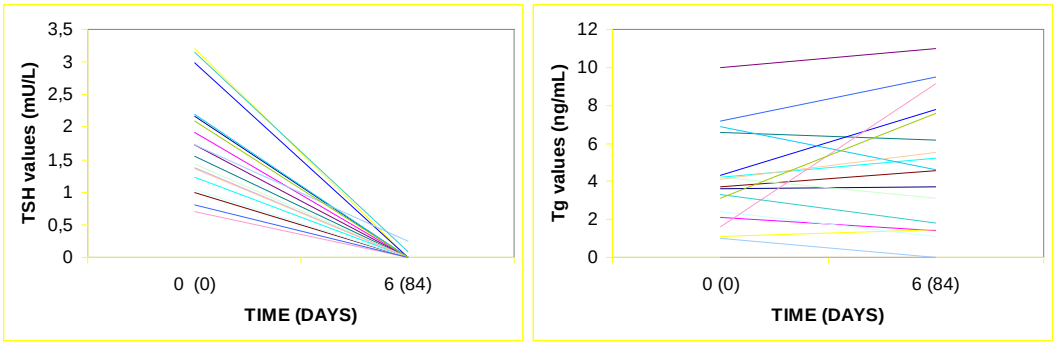

Figure 1. TSH and thyroglobulin values for each individual at the start and at the end of the protocol

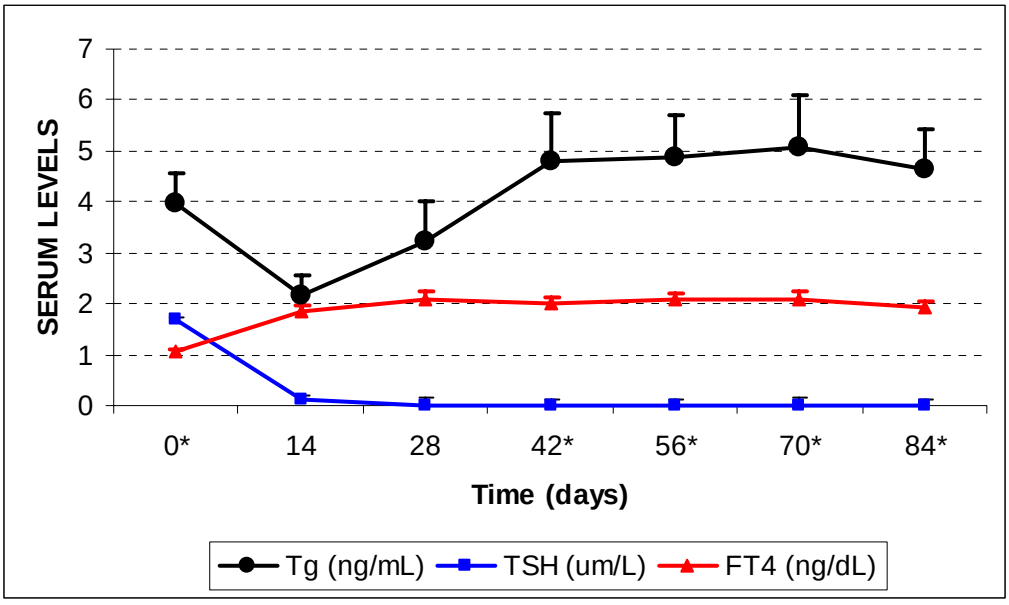

Figure 2. Behavior of the mean serum levels of Tg, TSH and FT4 during the 84 days of follow-up

* value of thyroglobulin statistically different from that in time 1 (14 days of L-T4 use) by Fisher´s exact test

4. CONCLUSÕES

CONCLUSÕES

Os achados deste estudo sugerem que os níveis séricos de Tg não sofrem alteração significativa em indivíduos eutiroidianos que usam L-tiroxina por um período de 84 dias.

Como visto, existem dois principais mecanismos de captação de Tg à partir do colóide a pinocitose e o mediado por megalina. A supressão do TSH provavelmente "desliga" ambos. Porém, ainda existe uma quantidade de megalina ja expressa e presente na membrana apical do tirócito. Esta ainda pode se ligar à Tg e promover sua transcitose até secretá-la intacta pela membrana celular basolateral.

Por esta hipótese, este é um processo auto-limitado; portanto, se os voluntários tivessem utilizado as mesmas doses de L-tiroxina por um período mais longo de tempo, provavelmente toda a megalina preformada seria gasta e os níveis séricos de Tg tornar-se-iam indetectáveis (corroborando os achados dos estudos descritos sobre tirotoxicose factícia).

Esta teoria é de difícil comprovação. A quantidade de Tg disponível em cada amostra de soro coletado é muito pequena para permitir a purificação de material suficiente para a realização de análises físico-químicas diretas (Druetta e cols., 1998).

5. REFERÊNCIAS

REFERÊNCIAS

Arvan P, di Jeso B 2005 in Werner and Ingbar´s The Thyroid Fundamental and Clinical Text, eds Braverman LE & Utiger RD, Lippincott, Williams & Wilkins, Philadelphia, pp 77-95

Baas F, Bikker H, van Kessel AG, Melsert R, Pearson PL, de Vijlder JJM, van Ommen GJB 1985 The human thyroglobulin gene: a polymorphic marker localized distal to c-myc on chromosome 8 band q 24. Human Genetics 69: 138-145

Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF e cols. 2003 National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 13: 3-126

Bernier-Valentin F, Kostrouch Z, Rabilloud R, Munari-Silem Y, Rousset B 1990 Coated vesicles from thyroid cells carry iodinated thyroglobulin molecules: first indication for an internalization of the thyroid prohormone via a mechanism of receptor-mediated endocytosis. J Biol Chem 265: 17373-17380

Bogazzi F, Bartalena L, Scarcello G, Campomori A, Rossi G, Martino E 1999 The age of patients with thyrotoxicosis factitia in Italy from 1973 to 1996. J Endocrinol Invest 22: 128-133

Carrasco N 2005 Thyroid iodine transport. in Werner and Ingbar´s The Thyroid Fundamental and Clinical Text, eds Braverman LE & Utiger RD, Lippincott, Williams & Wilkins, Philadelphia, pp 37-76

De Felice M, Di Lauro R 2004 Thyroid development and thyroid disgenesis. Endocr Rev 25: 722-746

De La Vieja A, Dohan O, Levy O, Carrasco N 2000 Molecular analysis of the sodium/iodide symporter: impact on thyroid and extrathyroid pathophysiology. Physiol Rev 80: 1083-1105

Druetta L, Croizet K, Bornet H, Rousset B 1998 Analysis of the molecular form of serum thyroglobulin from patients with Graves' disease, subacute thyroiditis or diferentiated thyroid cancer by velocity sedimentation sucrose gradient and Western blot. Eur J Endocrinol 139: 498-507

Druetta L, Bornet H, Sassolas G, Rousset B 1999 Identification of thyroid hormone residues on serum thyroglobulin: a clue to the source of circulating thyroglobulin in thyroid diseases. Eur J Endocrinol 140: 457-467

Eskandari S, Loo DDF, Dai G, Levy O, Wright EW, Carrasco N 1997 Thyroid Na^+/I^- symporter: mechanism, stoichiometry and specificity. J Biol Chem 272: 27230-27238

Hjort T 1961 Determination of serum thyroglobulin by a haemagglutination-inhibition test. Lancet i 1262-1264

Kostrouch Z, Munari-Silem Y, Rajas F, Bernier-Valentin F, Rousset B 1991 Thyroglobulin internalized by thyrocytes.passes through early and late endosomes. Endocrinology 129: 2202-2211

Larsen PR, Davies TF, Schlumberger M, Hay ID 2003 Thyroid Physiology and Diagnostic Evaluation of patients with thyroid disorders, in Williams Textbook of Endocrinology, eds Larsen PR, Kronenberg HM, Melmed S & Polonsky KS, Saunders (Elsevier science), Philadelphia, p 331-373

Lisi S, Pinchera A, McCluskey RT, Willnow TE, Refetoff S, Vitti P e cols. 2003 Preferential megalin-mediated transcytosis of low-hormonogenic thyroglobulin: a

control mechanism for thyroid hormone release. Proc Natl Acad Sci 100: 14858-14863

Lisi S, Segnani C, Mattii L, Botta R, Marcocci C, Dolfi A e cols. 2005 Thyroid dusfunction in megalin deficient mice. Mol Cel Endocrinol 236: 43-47

Marinò M, Chiovato L, Mitsiades N, Latrofa F, Andrews D, Tseleni-Balafouta S e cols 2000 Circulating thyroglobulin transcytosed by thyroid cells is complexed with secretory components of its endocytic receptor megalin. J Clin Endocrinol Metab 85: 3458-3467

Marinò M, McCluskey RT 2000 Role of thyroglobulin endocytic pathways in control of thyroid hormone release. Am J Physiol Cell Physiol 279: C1295-1306

Marinò M, Pinchera A, McCluskey RT, Chiovato L 2001 Megalin in thyroid physiology and pathology. Thyroid 11: 47-56

Mariotti S, Martino E, Cupini C, Lari R, Giani C, Baschieri L, Pinchera A 1982 Low serum thyroglobulin as a clue to the diagnosis of thyrotoxicosis factitia. N Engl J Med 307: 410-412

Mazzaferri EL 1999 NCCN thyroid carcinoma practice guidelines. 1999 oncology, volume 13, supplement no. 11A, NCCN procedings. 13: 391-442, www.nccn.org/guidelines.htm

Mazzaferri EL, Kloos RT 2001 Clinical review: current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab 86: 1447-1463

Ohmyia Y, Hayashi H, Kondo T, Kondo Y 1990 Location of dehydroalanine residues in the amino acid sequence of bovine thyroglobulin: identification of

"donor" thyrosine sites for hormonogenesis in thyroglobulin. J Biol Chem 265: 9066-9071

Ohno M, Zannini M, Levy O, Carrasco N, Di Lauro R 1999 The paired-domain transcription factor Pax8 binds to the up-stream enhancer of the rat sodium/iodide symporter gene and participates in both thyroid specific and cyclic-AMP-dependent transcription. Mol Cell Biol 19: 2051-2060

Ohye H, Fukata S, Kanoh M, Kubota S, Kuma K, Miyauchi A, Sugawara M 2005 Thyrotoxicosis caused by weight-reducing herbal medicines. Arch Intern Med 165: 831-834

Preisner CM, O´Kane DJ, Singh RJ, Morris JC, Grebe KSG 2003 Phantoms in the assay tube: heterophile antibody interpherences in serum thyroglobulin assays. J Clin Endocrinol Metab 88: 3069-3074

Schlumberger M, Pacini F, Wiersinga WM, Toft A, Smii JW, Sanchez-Franco F e cols. 2004 Follow-up and management of differentiated thyroid carcinoma: a European perspective in clinical practice. Eur J Endocrinol 151: 539-548

Spencer CA, Wang CC 1995 Thyroglobulin measurement, techniques, clinical benefits, and pitfalls. Endocrinol Metab Clin N Am 24: 841-863

Spencer CA, Takeuchi M, Kazarosyan M 1996 Current status and performance goals for serum thyroglobulin assays. Clin Chem 42: 164-173

Spencer CA 2004 Challenges of thyroglobulin (Tg) measurement in the presence of Tg autoantibodies (TgAb). J Clin Endocrinol Metab 89: 3702-3704

Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti JS 2005 Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the

management of patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 90: 5566-5575

Taurog A, Dorris ML, Doerge DL 1996 Mechanism of simultaneous iodination and coupling catalysed by thyroid peroxidase. Arch Biochem Biophys 330: 24-32

Torréns J, Burch HB 2001 Serum thyroglobulin measurement. Endocrinol Metab Clin N Am 30: 429-67

Van Herle AJ, Vassart G, Dumont JE 1979 Control of thyroglobulin synthesis and secretion (part 2). N Engl J Med 301: 307-314