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ORIGINAL ARTICLE

Thyroid Function Tests in Nonthyroidal Illness: Correction by Mathematical Method

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Diagnostic confusion results when subnormal free T4 values are reported in nonthyroidal illness (NTI) when a variety of free thyroxine index or analogue techniques are used to estimate free thyroxine levels. We tried to correct the changes in free thyroxine assessments by a mathematical method in nonthyroidal illness (NTI). Serum albumin was used to correct the measured hormone concentration by mathematical formulas.

Materials and Methods: The study included 56 patients with acute and chronic systemic illnesses and control groups. Total T4 (TT4), total T3 (TT3), free T4 and free T3 by analogue method (FT4A and FT3A), free T4 by back titration (FT4B), TSH-IRMA, reverse T3, T3 Resin Uptake (T3RU), TBG, albumin and total serum proteins (TSP) were measured. Free T4 index (FT4I) and T4/TBG ratio (T4TBGR) were calculated. Mathematical correction for each hormone was done through equations based on patient's original hormone level and albumin concentration. As an example, the formula for correction of FT4A was:

expected FT4A = (patient's FT4A) - X (patient's albumin) + Y;

X = 1.11 × [(mean FT4A in normal subjects) ÷ (mean albumin in normal subjects)];

Y = 1.11 × (mean FT4A in normal subjects).

Results: The decrease in albumin and TSP resulted in a decrease in TT4 and TT3, FT4A and FT3A in NTI, but it had no significant effect on FT4B. Mathematical correction resulted in an increase in sensitivity of FT4A from 55.4 to 96.4%, FT4B from 94.6 to 100%, T4TBGR from 80.4 to 98.2%, and FT4I from 69.6 to 100%, in differentiating NTI from hypothyroidism. The specificity of tests remained unchanged. The corrections did not affect normal, hypothyroid, and hyperthyroid controls.

Conclusions: Mathematical correction increased sensitivity of tests, which assess free T4 directly or indirectly, in differentiating NTI from hypothyroidism. NTI has been reported as the most common cause of errors reported in thyroid function tests so mathematical correction could significantly increase overall accuracy of free T4 assessments.

Key Words: Nonthyroidal illness, NTI, Sick euthyroid syndrome, SES, Free T4, Free thyroxine index, Albumin, Mathematical correction

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Introduction

Nonthyroidal illness (NTI) is frequently accompanied by alterations in circulating thyroid hormone concentrations, despite patients remaining clinically euthyroid.[1] Although some NTI patients may indeed be hypothyroid,[2] routine assessment of thyroid function in acutely hospitalized patients has been questioned, because of low specificity

and poor positive predictive value.[3] We studied the effect of decrease in serum protein concentrations on thyroid function tests. Some previous studies demonstrated a correlation between low serum T3 and serum albumin in NTI patients.[4,5] We tried to correct the effect of decreased serum albumin concentration on free T4 assessments by a mathematical method in order to improve their accuracy. The required variables for such correction are concentration of serum albumin and the measured hormone. Then a computer program could be used to apply our mathematical formulas to the measurements and produce a corrected result.

Materials and Methods

The study included 56 patients with acute or chronic systemic illnesses hospitalized at a university general hospital (Table 1). The control groups included thirty healthy adults, ten hypothyroid and nine hyperthyroid patients. The inclusion criteria for NTI were absence of clinical evidence of thyroid disease, and negative history for known medications effective on thyroid axis regulation. Diagnoses in the patients admitted to the study included a wide variety of nonthyroidal illnesses such as gastrointestinal, liver, cardiovascular, pulmonary and cerebral disease, renal insufficiency, diabetes mellitus and cancer.

Malignant diseases, including adenocarcinomas and lymphomas, were the most frequent. The age of patients ranged between 12 and 82 years. The patients had no evidence of thyroid disease during their hospital stay. FT4B and TSH in combination at admission time and follow up were used to rule out thyroid disease.

Table 1. Primary illness in 57 patients with nonthyroidal illness

Primary illness	No of patients
Malignancy	18
Gastrointestinal disease	13
Renal disease	12
Uncontrolled diabetes mellitus	5
Respiratory disease	3
Cardiovascular disease	3
Cerebrovascular disease	3

The hormonal measurements included Coat-A-Count DPC RIA total T4 (TT4), total T3 (TT3), and T3 resin uptake (T3RU), GammaCoat Clinical Assay RIA free T4 by analogue method (FT4A), GammaCoat Clinical Assays RIA free T4 by back titration (FT4B), and also free T3 by analogue method (FT3A), Coat-A-Count DPC IRMA TSH, and reverse T3by RIA (Serono Diagnostica).

The measurements of serum proteins included total serum proteins (TSP) by refractometry method and albumin by quantitative agarose gel electrophoresis (REP, stained by Panceau REP). Thyroxine binding globulin (TBG) was measured by Gammadab (Clinical Assays, RIA). The standard calculations included: free T4 index (FT4I) and T4/TBG ratio (T4TBGR). We tried to correct the effect of alterations in serum albumin concentrations on total and free thyroxine assessments by novel mathematical calculations. The following equations were used for calculation of expected free T4 assays (eFT4A and eFT4B) and expected free T4 indirect assessments (eT4TBGR and eFT4I) independent of albumin concentration changes:

eFT4A = FT4A - 0.36 Albumin + 1.48
eFT4B = FT4B - 0.16 Albumin + 0.66
eT4TBGR = (T4/TBG) -0.61 Albumin+2.5
eFT4I = T4 (T3RU) -0.66 Albumin+2.71

Mathematical Basis of Calculations:

The mathematical basis for calculations of the expected values was based on the following equation:

Equation 1: *Expected Hormone Level* = *A* × *(Hormone level)* + *B* × *(Albumin)* + *C*. A, B, and C are three unknown variables. For calculation of these factors we need three equations. We used three "clear assumptions for making these equations:

The first assumption is that in an assumed normal case with hormone level equal to "mean normal hormone level of normal control group (NH)" and albumin equal to "mean normal albumin of normal control group (NA)", the expected hormone level should be equal to "expected hormone level of normal control group". The second assumption is that in another assumed case with hormone level equal to "zero", and albumin equal to "mean normal albumin of normal control group", the expected hormone level should be zero.

The third assumption is that in a case with hormone level equal to "mean hormone level of low albumin group (LH)" and albumin equal to "mean serum albumin of low albumin group (LA)", expected hormone level should be equal to "expected hormone level in low albumin group(EH)".

First equation: $NH = A \times (NH) + B \times (NA) + C$

Second equation: $0 = A \times (0) + B \times (NA) + C$

Third equation: $EH = A \times (LH) + B \times (LA) + C$

In the third equation we put the value of mean hormone level in normal control group (NH) instead of expected hormone level in low albumin group (EH) in case of free T4 assessments. The assumption was that the actual free T4 level should be normal in NTI. Solving the above three linear equations in three unknowns, it ensues:

$A = 1$;

$B = -(NH - LH) \div (NA - LA)$ and

$C = NA \times [(NH - LH) \div (NA - LA)]$.

By calculating A, B and C from the above equation based on normal and NTI groups data and placing them in Formula 1, the expected values for free T4 (eFT4A, eFT4B, eT4TBGR, eFT4I) are found.

Results

The frequency of normal T3 and normal T4 in patients was 16%, low T3 and normal T4 or "low T3 state" was 37.5%, and low T3 and low T4 or "low T3-T4 state" was 46.5%. The results of free hormone assessments and new mathematically corrected indices are presented in Table 2.

FT4A was below the normal range in 44.6%, FT4B in 5.4%, TT4 in 46.4%, FT4I in 30.4%, and T4TBGR in 19.6% of NTI cases. In 2 out of 3 patients with decreased FT4B the values were borderline low (0.81, and 0.87 ng/dL). In the third patient it was 0.69 ng/dL. The sensitivity and specificity of free T4 assessments in differentiating NTI from hypothyroidism, before and after mathematical correction are demonstrated in

Table 2. The results of free T4 assessments before and after mathematical correction by serum albumin and their sensitivity and specificity in differentiating NTI from hypothyroidism.

	Normal controls		NTI	Hypothyroid	Hyperthyroid	Sensitivity	Specificity
FT4A (ng/dL)							
Before	0.72-1.94*	1.33±0.31†	0.78±0.38†	0.36±0.31†	4.53±2.62†	55.4‡	90‡
After	0.62-2.04	1.33±0.36	1.33±0.37	0.3±0.33	4.6±2.62	96.4	90
FT4B (ng/dL)							
Before	0.87-2.33	1.6±0.37	1.35±0.3	0.34±0.21	3.1±0.5	94.6	100
After	0.84-2.38	1.6±0.39	1.6±0.3	0.31±0.21	3.1±0.5	100	100
T4TBGR							
Before	1.84-5.38	3.61±0.9	2.68±1.2	0.86±0.57	8.88±1.75	80.4	100
After	1.71-5.52	3.61±0.97	3.61±1.27	0.75±0.58	9±1.71	98.2	100
FT4I							
Before	1.37-3.73	2.55±0.6	1.54±0.43	0.54±0.21	6.53±1.12	69.6	100
After	1.16-3.94	2.55±0.71	2.55±0.51	0.43±0.44	6.67±1.12	100	100

* Range † Mean ± SD ‡ Percent

Table 2. We also used TSP instead of albumin and got equivalent results. Mathematical corrections had no significant effect on normal controls, hyperthyroid and hypothyroid patients.

Mean and standard deviation of serum albumin was 4.11±0.47 g/L in normal controls, and 2.58±0.75 g/L in NTI. Albumin was below the normal in 78.6%, TSP in 80.4%, and TBG in 14.3% of NTI. Albumin and TSP decreased significantly in NTI in comparison to control groups ($p<0.001$). Serum TBG level had no significant difference between the NTI and control groups. The results of correlations between serum proteins and thyroid function tests are illustrated in Tables 3 and 4, and Fig. 1.

Reverse T3 had a significant increase in NTI compared to normal controls ($p<0.01$). It was in the normal range in 32.1% of NTI. Reverse T3 was increased in malignancies, diabetes, and cerebrovascular accidents but it was in the upper normal range in CRF and cardiopulmonary patients.

TT3 was below the normal in 84% and FT3A in 58.9% of NTI.

TSH was in the normal range in 94.6% of NTI patients and varied from 4.97 to 8.6 mU/L in three patients (5.4%). The lowest TSH value in NTI was 0.39 mU/L. TSH in the present study did not change significantly in NTI, but its deviation from the mean value was greater than to the healthy controls (mean±SD was 2.08±1.95 in NTI versus 1.85±0.59 in healthy controls).

Discussion

Nonthyroidal illness is probably a more common cause of abnormalities in the serum concentration of thyroid hormones than intrinsic thyroid disease. The prevalence of one or more abnormalities of thyroid function tests in patients with acute medical illnesses has been reported from 40% to 70%.[6,7] Although thyroid function test alterations occur in nonthyroidal illnesses,[8-11] the result of in vitro estimation of free T4 does not always correlate with the transfer of T4 to tissues.[12] Some mechanisms are involved in incompletely understood these Abnormalities.[13]

Table 3. The correlation among serum proteins and thyroid hormone assays in normal control group.

	TT4	FT4A	FT4B	TT3	FT3A
Albumin	0.15*	0.02	-0.20	0.38†	0.17
TSP	0.28	-0.47‡	-0.12	0.11	0.13
TBG	0.28	-0.47	-0.28	0.11	-0.12

* Numbers represent r (Correlation coefficient) † P<0.05 ‡ P<0.01

Table 4. The correlation among serum proteins and thyroid hormone assays in NTI.*

	TT4	FT4A	FT4B	TT3	FT3A
Albumin	0.49*†	0.39‡	0.23	0.71†	0.71†
TSP	0.46†	-0.48†	0	0.50†	0.45†
TBG	0.46†	0.21	0.04	0.40†	0.30‡

* Numbers represent r (Correlation coefficient) † P<0.001 ‡ P<0.05

Measurement of free thyroxine by analogue techniques or free thyroxine index result in subnormal free T4 levels because of technical limitations.[14] Thyroid hormone binding proteins, primarily albumin, affect free T4 measurements in analogue methods.[15-25] In these methods binding of T4 analogue to albumin is responsible for the erroneous results, falsely low, when the albumin concentration is markedly decreased. Hence in complicated clinical situations such as severe NTI, decrease in serum albumin concentration results in less binding of tracer by albumin and thus produces artifactually lowered free T4 measurements. Figure 1 demonstrates the correlation between serum albumin and FT4A and FT4B. These problems have not yet been solved despite attempts to improve the RIA system by adding blockers that chemically inhibit the analogue-albumin binding.[26,27] In the present study the adverse effects of decrease in serum albumin concentration on free T4 assessments were corrected by a mathematical method.

Among the four free T4 assessments the FT4A had lowest accuracy. Its sensitivity in differentiating NTI from hypothyroidism rose from 55.4% to 96.4% after correction. Values

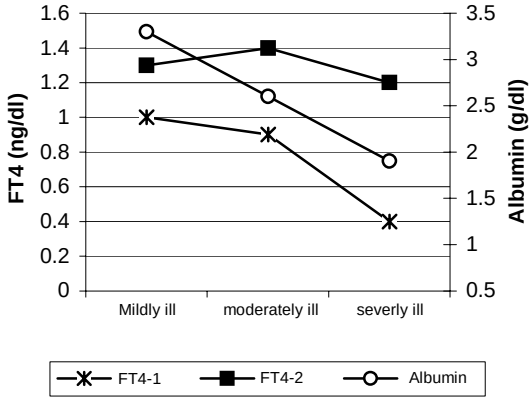

Fig. 1. Correlation between mean serum albumin and FT4 1-step and 2- step in three groups of patients with NTI

obtained by FT4B are comparable to those determined with the direct methods.[28,29] FT4B had the least correlation with fluctuations of serum proteins in this study. In three patients with low FT4B, TSH values were normal. Its sensitivity was 94.6% and increased to 100% after correction. Total T4 had significant correlation with albumin in NTI. Sensitivity of T4/TBG ratio (T4TBGR) and free thyroxine index (FTI) increased from 80.4% and 69.6% to 98.2% and 100% after correction, respectively. The specificity of all four tests remained unchanged. Free T3 by ultra filtration remains within normal range in NTI even in the severely ill, low T3-low T4 group.[30,31] It decreases with increasing severity of underlying disease in NTI.[32] The results of FT3A and TT3 were below the normal range in 58.9% and 83.9% of NTI, respectively. Cytokines have been implicated in the pathogenesis of low T3 syndrome during illness.[32,33] There is a strong negative relationship between serum T3 and serum interlukin-6 (IL-6) in NTI.[32] TGF-beta1 could play a role in the pathogenesis of some modifications of thyroid function observed in patients with nonthyroidal illnesses.[34]

Albumin, TSP and TBG had no significant correlation with thyroid function tests in the normal control group. The exception was TSP with FT4A ($r=-0.47$, $p<0.01$), and albumin with TT3 ($r=-0.38$, $p<0.05$). In NTI the correlations between serum proteins and thyroid function tests were statistically significant in most instances (Table 4). Although TBG concentration was normal in NTI, it had been shown that decline in total T4 is partly due to a decrease in T4 binding to TBG.[35] Serum TBG showed no significant difference between NTI and control groups in the present study.

TSH usually remains unchanged in NTI.[36] The causes of transient alterations in serum TSH values in some NTI are multifactorial, however low TSH values are most frequently

found in patients who have received pharmacological doses of glucocorticoids or dopamine.[37]

Each laboratory should use its own mean free hormone level and albumin concentration in a normal population as a reference for mathematical correction. The following are simplified formulas for calculating expected hormone values:

eFT4A = (patient's FT4A) - X (patient's albumin) + Y, where:
X = 1.11 [(mean FT4A in normal subjects) ÷ (mean albumin in normal subjects)], and
Y = 1.11 (mean FT4A in normal subjects)

eFT4B = (patient's FT4B) - X (patient's albumin) + Y, where:
X = 0.41 [(mean FT4B in normal subjects) ÷ (mean albumin in normal subjects)]
Y = 0.41 (mean FT4B in normal subjects)

eT4TBGR = [(patient's T4) ÷ (patient's TBG)] - X (patient's albumin) + Y, where:
X = 0.69 [(mean T4TBGR in normal subjects) ÷ (mean albumin in normal subjects)] Y = 0.69 (mean T4TBGR in normal subjects)

eFT4I = (patient's T4) (patient's T3RU) – X (patient's albumin) + Y, where:
X = 1.06 [(mean FT4I in normal subjects) ÷ (mean albumin in normal subjects)]
Y = 1.06 (mean FT4I in normal subjects)

Our mathematical correction increased sensitivity of tests, which assess free T4 directly or indirectly, in differentiating NTI from hypothyroidism. Its improved accuracy is comparable to the method of combining total T4 and T3RU to produce more accurate free T4 index (FTI). We suggest mathematical correction as a practical way to improve the results of thyroid function tests in NTI. This method is an example of application of mathematics in medicine.

References

1. Davies PH, Black EG, Sheppard MC, Franklin JA. Relation between serum interleukin-6 and thyroid hormone concentrations in 270 hospital in-patients with non-thyroidal illness. Clin Endocrinol (oxf) 1996; 44: 199-205.
2. Chopra IJ. Clinical review 86: Euthyroid sick syndrome: is it a misnomer? J Clin Endocrinol Metab1997; 82(2): 329-34.
3. Stockigt JR. Guidelines for diagnosis and monitoring of thyroid disease: nonthyroidal illness. Clin Chem 1996; 42: 188-92.
4. Sapin R, Schlienger JL, Kaltenbach G, Gasser F, Christofides Nroul G, et al. Determination of free triiodothyronine by six different methods in patients with non-thyroidal illness and patients treated with amiodarone. Ann Clin Biochem 1995; 32 (pt3):314-24.
5. Casako G, Zweig MH, Glickman J, Ruddel M, Kestner J. Direct and indirect techniques for free thyroxin compared in patient with nonthyroidal illness. Effect of prealbumin, albumin, and thyroxin-binding globulin. Clin Chem 1989; 35(8): 1655-62.
6. Bermudez F, Surks M, Oppenheimer JH. High incidence of decreased serum triiodothyronine concentration in patients with nonthyroidal disease. J Clin Endocrinol Metab 1975; 41:27-40.
7. Kaplan MM, Larsen PR, Crantz FR. Prevalence of abnormal thyroid function test results in patients with acute medical illnesses. Am J Med 1982; 72: 9-16.
8. Karga H, Papaioannou P, Venetsanou k, Papndroulaki F, Karaloizos L, Papaioannou G, et al. P. The role of cytokines and cortisol in the non-thyroidal illness syndrome following acute myocardial infarction. Eur J Endocrinol 2000; 142(3): 236-42.
9. Hein MD, Jackson IM. Review: thyroid function in psychiatric illness. Gen Hosp Psychiatry 1990;12(4);232-44
10. Kayima JK, Otieno LS, Gitau W, Mwai S. Thyroid hormone profiles in patients with chronic renal failure on conservative management and regular haemodialysis. East Afr Med J 1992; 69(6): 333-6.
11. Custro N, Scafidi V, Costanzo G, Notarbartolo A. Prospective study on thyroid function anomalies in severely ill patients. Ann Ital Med Int 1992; 7(1): 13-8.

12. Sarne DH, Refetoff S. measurement of thyroxin uptake from serum by cultured human hepatocytes as an index of thyroid status: reduced thyroxin uptake from serum of patients with nonthyroidal illness. J Clin Endocrinol Metab 1985; 61(6): 1046-52.
13. Santini F, Chiovato L, Bartalen, Lapi P, Palla R, Panichi V, et al. Study of serum 3,5,3'-triiodothyronine sulfate concentration in patients with systemic non-thyroidal illness. Eur J Endocrinol 1996; 134(1): 45-9.
14. Kaptein EM, Macintyre SS, Weiner JM, Spencer CA, Nicoloff JT. Free thyroxine estimations by in nonthyroidal illness: comparison of eight methods. J Clin Endocrinol Metab 1981; 52: 1073-77.
15. Nelson JC, Weiss RM, Wilcox RB. Underestimates of serum free thyroxine (T4) concentrations by free T4 immunoassays. J Clin Endocrinol Metab 1994; 79: 76-9.
16. Ekins R. Measurement of free hormones in blood. Endocr Rev 1990; 11: 5-46.
17. Rajatanavin R, Fournier L, DeCasimo D, Abreau C, Braverman LE. Elevated serum free thyroxine by thyroxine analogue radio-immunoassy in euthyroid patients with FDH. Ann Intern Med 1982; 97: 865-6.
18. Stockigt JR. Influence of altered plasma binding on free and total thyroid hormone levels. In: Albertini A, Ekins R, eds. Free hormone in blood. Amesterdam: Elsevier Biomed Press, 1982: 223-30.
19. Stockigt JR, Stevens V, White EL, Barlow JW. "Unbound analogue" radioimmunoassays for free thyroxine measure the albumin-bound hormone fraction. Clin Chem 1983; 29: 1408-10.
20. Ooi DS, Sorisky A. Effect of albumin on results of analogue-type assays for thyroxine. Clin Chem 1984; 30: 1109-10.
21. Csako G, Zwieg MH, Benson C, Ruddel M. On the albumin dependence of measurements of free thyrxoine. I. Technical performance of seven methods. Clin Chem 1986; 32: 108-15
22. Csako G, Zwieg MH, Benson C, Ruddel M. On the albumin dependence of measurements of free thyroxine. II. Patients with nonthyroidal illnesses. Clin Chem 1987; 33: 87-92.
23. Midgley JE, Sheehan CP, Christofides ND, Fry JE, Browning D, Mardell R. Concentrations of free thyroxine and albumin in serum in severe nonthyroidal illness: Assay artefacts and physiological influences. Clin Chem 1990; 36: 765-71.
24. Deam D, Goodwin M, Ratnaike S. Comparison of four methods for free thyroxine. Clin Chem 1991; 37: 569-72.
25. Ekins R. Validity of analogue free thyroxine immunoassays. Clin Chem 1987; 33: 2137-44.
26. Witherspoon LR, el Shami AS, Shuler SE, Neely H, Sonnemaker R, Gilbert SS, et al. Chemically blocked analog assays for free thyronines. I. The effect of chemical blockers on T4 analog and T4 binding by albumin and by thyroxin-binding globulin. Clin Chem 1988 Jan; 34(1):9-16.
27. Sakata S, Komaki T, Shiraki S, Kumasaki N, Iwata H. Effect of serum albumin concentration on free thyroxine (Amerlex FT4) values in healthy nonpregnant and pregnant women. Clin Chem Acta 1988; 176: 225-6.
28. Nuutila P, Koskinen P, Irjala K, Linko L, Kaihola HL, Eskola JU, et al. Two new two-step immunoassays for free thyroxin evaluated: solid-phase radioimmunoassay and time-resolved fluoroimmunoassay. Clin Chem 1990; 36: 1355-60.
29. Hay ID, Bayer MF, Kaplan MM, Klee GG, Larson PR, Spencer CA. American Thyroid Association Assessment of current free thyroid hormone and thyrotropin measurements and guidelines for future clinical assays. Clin Chem 1991;37: 2002-8.
30. Chopra IJ. Simultaneous measurement of free thyroxine and free 3,5,3'-triiodothyronine in undiluted serum by direct equilibrium dialysis / radioimmunoassay: evidence that free triiodothyronine and free thyroxine are normal in many patients with the low triiodothyronine syndrome. Thyroid 1998; 8(3):249-57.
31. Faber J, Kirkegaard C, Rasmussen B, Westh H, Busch Sorensen M. Pituitary-thyroid axis in cririca illness. J Clin Endocrinol Metab 1987; 65:315-20.
32. Boelen A, Platvoet-Ter Schiphorst MC, Wiersinga WM. Soluble cytokine receptors and the low 3,5,3'-triiodothyronine syndrome in patients with nonthyroidal disease. J Clin Endocrinol Metab 1995; 80:971-6.
33. Torpy DJ, Tsigos C, Lotsikas AJ, Defensor R, Chrousos GP, Papanicolaou DA. Acute and delayed effects of a single-dose injection of interleukin-6 on thyroid function in healthy humans. Metabolism 1998; 47(10):1289-93.

34. Corica F, Allegra A, Corsonello A, Buemi M, Rubino F, Bonanzinga S, et al. Increased transforming growth factor-beta1 plasma concentration is associated with high plasma 3,3',5'-tri-iodothyronine in elderly patients with nonthyroidal illnesses. Eur J Endocrinol 1998; 138: 47-50.
35. Kaptein EM, Greib DA, Spencer CA, Wheeler WS, Nicoloff JT. Thyroxine metabolism in the low thyroxine state of critical nonthyroidal illness. J Clin Endocrinol Metab 1981; 53: 764-71.
36. Wartosky L, Burman KD. Alterations in thyroid function in patients with systemic illness: the "euthyroid sick syndrome". Endocr Rev 1982; 3: 164-217.
37. Samuels MH, Luther M, Henry P, Ridgway EC. Effects of hydrocorsione on pulsatile pituitary glycoprotein secretion. J Clin Endocrinol Metab1994; 78: 211-15.