

The hypothalamic-pituitary-thyroid axis in subjects with subclinical thyroid diseases: The impact of the negative feedback mechanism

Paolo Falaschi, Antonio Martocchia, Antonella Proietti, Rosaria D'Urso, Serena Gargano, *Franco Culasso & Antonio Rocco

Chair of Internal Medicine and *Statistics, I and II Faculty of Medicine, University of Rome, "La Sapienza", ITALY.

Correspondence to: Prof. Paolo Falaschi,
Via di Tor Fiorenza 13, 00199 Rome, ITALY
TEL: +39-6-86211484,
FAX: +39-6-80345401,
E-MAIL: paolo.falaschi@uniroma1.it

Submitted: December 14, 2003

Accepted: April 23, 2004

Key words: **HPT axis; negative feedback; subclinical hypothyroidism; subclinical hyperthyroidism; regression analysis**

Neuroendocrinol Lett 2004; 25(4):292-296 NEL250404A09 Copyright © Neuroendocrinology Letters www.nel.edu

Abstract

OBJECTIVE: To evaluate the hypothalamus-pituitary-thyroid (HPT) axis in patients with subclinical thyroid dysfunction recently reported to have either symptoms or organ involvements with potential morbidity, in order to better differentiate these patients with respect to controls.

PATIENTS Sixtythree patients with subclinical hyperthyroidism (HyperT), 178 normal subjects (EuT) and 106 patients with subclinical hypothyroidism (HypoT) were enrolled; the groups presented normal thyroid hormone (FT4, FT3) levels and, respectively, reduced (HyperT), normal (EuT) and increased (HypoT) TSH levels. The negative feedback was simultaneously evaluated by multiple linear regression.

RESULTS: The mean TSH, FT4 and FT3 levels were significantly different in the three groups. The negative correlation between thyroid hormones (FT4 and FT3) and TSH secretion was significant ($p < 0.001$ in HyperT; $p < 0.01$ in EuT; $p < 0.000001$ in HypoT group). FT4 mostly contributed to the negative correlation with TSH.

The normal ranges of TSH values was accurately defined on the basis of the regression equation in the EuT group, due to the combining influence of both thyroid hormones (FT3 and FT4). No patient of the HyperT or HypoT group fell inside the range of estimated values of the normal group.

CONCLUSIONS: The HPT axis in patients with subclinical hyper- and hypo-thyroidism is significantly modified with respect to normal subjects. The status of the axis, as evaluated by the relationship between the three hormones (FT4, FT3, TSH) together considered, is characteristic of the normal or pathologic condition. A reliable method based on the regression analysis is proposed to correctly evaluate the status of the HPT axis.

Abbreviations

HPT	hypothalamic-pituitary-thyroid axis
HyperT	subclinical hyperthyroidism
EuT	normal subjects
HypoT	subclinical hypothyroidism
FT4	free thyroxine
FT3	free triiodothyronine
TSH	thyrotropin
RIA	radioimmunoassay
x, y, z	three variables of the linear regression analysis model
a, b	slopes of the linear regression analysis model
c	intercept of the linear regression analysis model
R	multiple correlation coefficient
DF	difference between esteemed and measured TSH value
SD	standard deviation
SE	standard error

Introduction

The screening with ultra-sensitive thyroid function tests allows the identification of a great number of patients with clinically inapparent thyroid dysfunctions [1]. Many efforts have been made to develop guidelines to assist clinicians in the management of such patients [2]. The diagnosis of subclinical hyper- and hypothyroidism is usually suggested when patients show normal free thyroid hormone levels and, respectively, undetectable or elevated TSH levels, in the absence of evident clinical symptomatology [2]. However, it has been recently reported that patients with subclinical thyroid dysfunctions may present either symptoms due to the disease or evidence of organ system involvement and potential morbidity [3–10]. Since clinical evidence suggests to consider the subjects with borderline thyroid abnormalities as patients at the initial stage of disease, the areas of uncertainty between the normal and abnormal function of hypothalamic-pituitary-thyroid (HPT) axis are matter of continue debate to achieve a more accurate diagnosis [8–10].

The aim of the study was to investigate the negative feedback mechanism of the HPT axis negative feedback in patients with subclinical thyroid dysfunction (either hyper- and hypo-thyroidism), in order to better differentiate these patients with respect to normal subjects.

Material and methods

Sixtythree consecutive patients with subclinical hyperthyroidism (HyperT group), 178 normal subjects (EuT group) and 106 patients with subclinical hypothyroidism (HypoT group) were enrolled in the study. All the patients were recruited in the outpatient of the Department of Internal Medicine. Subclinical hyper- and hypo-thyroidism were defined by the presence of normal thyroid hormone (FT4, FT3) levels and, respectively, reduced or increased TSH levels (with cut-off value of <0.04 and >3.8 $\mu\text{U/ml}$, respectively), in absence of clinically evident symptomatology. The normal subjects showed FT4, FT3 and TSH in the normal range of values; thyroid diseases or acute/chronic diseases (above all those involving the thyroid gland)

were excluded by the history and clinical examination. No patient was receiving L-T4 therapy.

Determination of FT4 and FT3 levels was performed by RIA (kit provided by IST-Medical Systems, Genoa – Italy) and TSH was determined by RIA (kit provided by Biochem Immunossystems, Milan – Italy). Normal range of FT4, FT3 and TSH was respectively 8.0–20.0 pg/ml, 1.4–4.4 pg/ml and 0.4–3.8 $\mu\text{U/ml}$; inter- and intra-assay coefficients of variation were respectively 5.2–8.9%, 5.8–9.2% for FT4 and FT3 and 4.2–7.8% for TSH.

Statistical analysis of the difference between mean hormonal values among groups was performed by the analysis of variance with Bonferroni's t-test procedure. $P < 0.05$ was assumed as significant.

The relationship between two variables (FT4 and FT3, FT4 and TSH, FT3 and TSH) was evaluated by linear regression analysis, in each group of subjects. Moreover, the negative feedback between the thyroid hormones (FT4 and FT3) and TSH was simultaneously evaluated by multiple linear regression. Since FT4 is the main secretory product of the thyroid gland and the most part of FT3 derives from the peripheral conversion of FT4 to FT3 and considering that both hormones participate in negative feedback mechanism on TSH secretion, the following three-variable model was used: $z = ax + by + c$, where x was FT4, y was FT3, z was TSH, a and b were the slopes and c was the intercept. The result of the multiple linear regression was plotted in a three-axis graphic, being TSH values on a logarithmic scale. For the EuT group, the solid volume included the 95.0% of the data (the volume being obtained moving the regression line in the three axis, until the 5.0% of the data fell outside the normal range).

In the multiple regression analysis, the multiple correlation coefficient (R) and the variance analysis indicating the F value and the significance level were calculated and applied. The single contribution of each regression parameter to the multiple regression analysis was evaluated by the t-test: $t = a/SE_a$, $t = b/SE_b$, $t = c/SE_c$ (SE = standard error). The 95.0% confidence interval of the regression parameters was reported. The difference (DF) between the esteemed TSH value (as consequence of the substitution of FT3 and FT4 values related to the subject in the corresponding equation) and the measured TSH value was also evaluated. The range of the difference was calculated as the interval between the mean ± 2 standard deviation (SD).

Results

Mean age (\pm SD) and female:male (F:M) ratio in the three groups were, respectively: 49.82 \pm 8.20 ys (F:M=42:21) in HyperT, 48.39 \pm 14.56 ys (F:M=101:77) in EuT and 45.23 \pm 14.96 ys (F:M=77:29) in HypoT group. The hormonal (FT3, FT4 and TSH) levels were, respectively: 3.01 \pm 0.69 pg/ml, 15.42 \pm 2.45 pg/ml and 0.20 \pm 0.07 $\mu\text{U/ml}$ in HyperT (m \pm SD); 2.66 \pm 0.61 pg/ml, 14.04 \pm 2.22 pg/ml and 1.79 \pm 0.73 $\mu\text{U/ml}$ in EuT (m \pm SD); 2.45 \pm 0.61 pg/ml, 12.80 \pm 2.59 pg/ml and

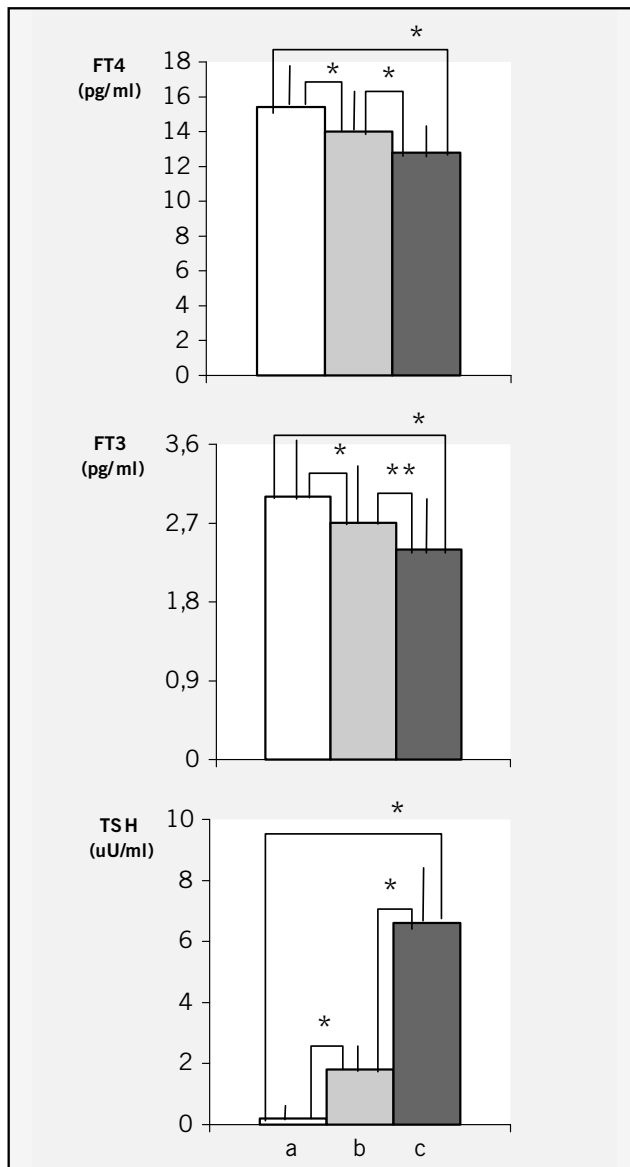


Fig.1. Hormonal levels in patients with subclinical thyroid dysfunctions and in controls (mean \pm standard deviation).
 a = subclinical hyperthyroidism (n=63)
 b = control subjects (n=178)
 c = subclinical hypothyroidism (n=106)
 *p<0.001, **p<0.01

6.58 \pm 2.47 μ U/ml in HypoT (m \pm SD) FT3, FT4 and TSH: HyperT vs EuT, EuT vs HypoT and HyperT vs HypoT all p<0.001, except EuT vs HypoT FT3 (p<0.01) (fig.1).

As expected, the linear regression analysis showed that, in HyperT group FT4 was positively correlated to FT3 (p<0.05), FT4 were negatively correlated to TSH (p<0.001) and FT3 were negatively correlated to TSH (p<0.001). Similar results were present in the other two groups, respectively: in EuT group, FT4 was positively correlated to FT3 (p<0.01), FT4 was negatively correlated to TSH (p<0.02) and FT3 was negatively correlated to TSH (p<0.02); in HypoT group, FT4 was positively correlated to FT3 (p<0.01), FT4 was negatively correlated to TSH (p<0.001) and FT3 was negatively correlated to TSH (p<0.001) (data not shown). The multiple regression analysis allowed us to simultaneously evaluate the combined effects of both FT3 and FT4 on TSH secretion. A statistical negative significant correlation was found between thyroid hormones (FT4 and FT3) and TSH secretion, as represented by the respective equations, in the three groups (tab.1).

The regression line in HyperT, EuT and HypoT group was reported in fig.2.

The mean (\pm SD) difference between the esteemed and measured TSH (see material and methods) in the EuT group was 0.01 \pm 0.71 μ U/ml, with a range (mean \pm 2SD) of -1.41 \leq DF \leq +1.43 μ U/ml.

The FT4 and FT3 values of the subjects of HyperT and HypoT group were substituted in the equation of the EuT group. No patient with subclinical hyper- or hypo-thyroidism fell inside the above indicated DF range of EuT group. In particular, all estimated TSH values of HyperT patients resulted above the upper limit of the range (DF from the measured TSH > +1.43 μ U/ml) (HyperT TSH was over-estimated by the EuT equation) and all patients of the HypoT group resulted under the lower limit of the range (DF from the measured TSH < -1.41 μ U/ml) (HypoT TSH was under-estimated by the EuT equation). Six normal subjects with measured TSH levels between 3.1–3.7 μ U/ml exceeded the DF range (DF < -1.41 μ U/ml).

Table 1 . The equations obtained by the multiple regression analysis between FT4, FT3 and TSH values, in normal subjects (EuT group) and in patients with subclinical hyper- (HyperT group) and hypo-thyroidism (HypoT group).

HyperT group:	EuT group:	HypoT group:
1) Equation: TSH=-0.008·FT4-0.030·FT3+0.42	TSH=-0.05·FT4-0.21·FT3+3.06	TSH=-0.31·FT4-1.23·FT3+13.54
2) Multiple regression analysis: R= -0.46, F=8.04, p<0.001 95% confidence interval for: a= -0.015/-0.002 b= -0.054/-0.005 c= 0.308/0.535	R= -0.25, F=6.07, p<0.01 95% confidence interval for: a= -0.099/-0.003 b= -0.384/-0.031 c= 2.310/3.814	R= -0.50, F=17.00, p<0.000001 95% confidence interval for: a= -0.015/-0.002 b= -0.054/-0.005 c= 0.308/0.535
3) The single contribution of each partial regression parameter: t _a = -2.461 p<0.02 t _b = -2.439 p<0.02 t _c = 7.432 p<1·E ⁻⁹	t _a = -2.318 p<0.05 t _b = -2.086 p<0.05 t _c = 8.036 p<1·E ⁻¹²	t _a = -3.647 p<0.001 t _b = -3.408 p<0.001 t _c = 11.119 p<1·E ⁻¹⁸

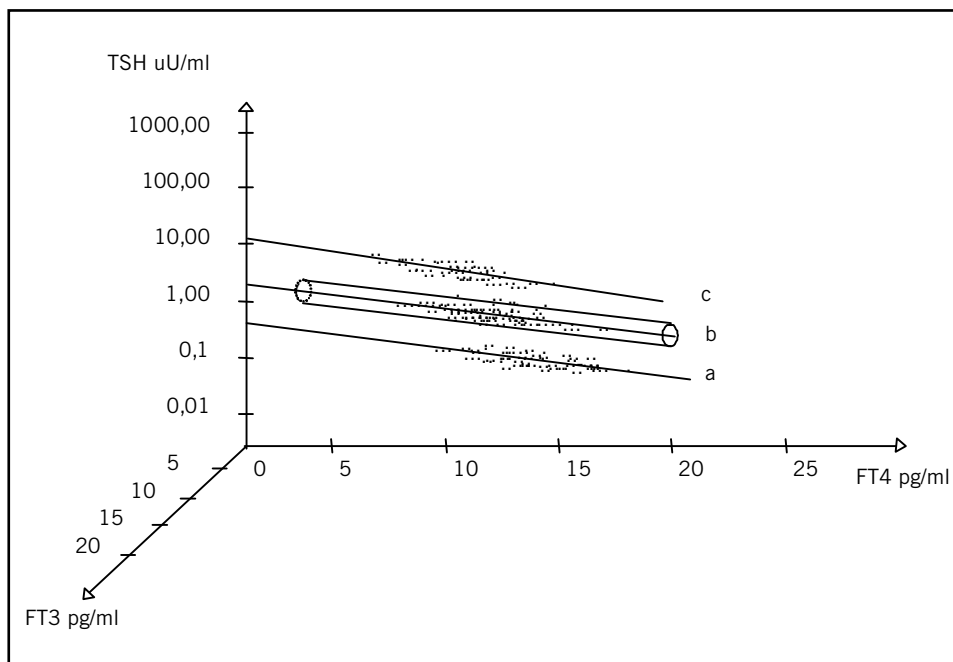


Fig.2. Thyroid hormone negative feedback in patients with subclinical thyroid dysfunction and in controls.

a = subclinical hyperthyroidism (n=63)
 b = control subjects (n=178)
 c = subclinical hypothyroidism (n=106)

Discussion

The study documented significant alterations of the hypothalamic-pituitary-thyroid axis in patients with subclinical either hyper- and hypo-thyroidism. In fact, besides the significant TSH difference between the patient groups and the normal subjects, both mean FT4 and FT3 levels were significantly different among groups. When HPT negative feedback is disrupted (as evidenced by the suppressed or elevated TSH levels), rather than compensate, the status of thyroid hormone secretion (as revealed by FT4 and FT3 levels) seems to be already significantly modified in the opposite way. The modified pituitary TSH secretion does not actually compensate the thyroid dysfunctions and the presence of the thyroid hormone levels in the normal range seems to be an insufficient, incomplete and, for some instance, uncorrect criteria. Similar results have been described in studies investigating the presence of organ system involvement and potential morbidity in the patients with subclinical thyroid dysfunctions [5, 7]. According to this evidence, in a recent paper, patients with subclinical hyperthyroidism had the same relative risk of atrial fibrillation compared to overt hyperthyroidism [8]. Indeed, a more accurate definition of normal HPT axis function may be the correct relationship between FT4, FT3 and TSH levels, as evaluated by the regression equations. The difference between the measured and esteemed TSH may be proposed as a simple and reliable method to evaluate and quantify the status of HPT axis feedback. The regression equation in the control group may give additional informations about the TSH levels in normal condition. In fact, the above described equation reveals that the esteemed TSH level of 3.06 μ U/ml (with a 95% confidence interval of 2.31–3.81 μ U/ml) corresponds to a FT4 and FT3 secretion that paradoxically approximates the zero level. It is noteworthy to point out

that even if the 3.06 μ U/ml value of TSH is within the normal range of reference, as provided by the commercial kits, it appears to be unequivocally not a normal value. At the same time, the 1.11 μ U/ml value of TSH (comprised in the normal reference range) (with a 95% confidence interval of 0.36–1.86 μ U/ml) identifies a FT4 value of 20.1 pg/ml and a FT3 value of 4.5 pg/ml, that are beyond the upper limit of normal range and, for this reason, not in the normal value.

In conclusion, the HPT axis in patients with clinical hyper- and hypo-thyroidism is modified with respect to normal subjects. The status of the axis, as evaluated by the relationship between the three hormones (FT4, FT3 and TSH) together considered, appears to be characteristic of the normal or pathologic condition. The clinical spectrum from the normal to the subclinical thyroid hyper- or hypo-function seems to be discontinuous rather continuous. The absence of overlap between groups would individualize a particular area that we could define “inapparent thyroid dysfunction”.

The criteria usually employed in clinical evaluation of subclinical thyroid dysfunction are not accurate. The difference between the measured and esteemed TSH (by the means of regression equation) may be a simple and reliable method to correctly evaluate and quantify the status of HPT axis feedback, in particular, in subjects with TSH values in the upper limit of normal ranges (>3 μ U/ml). Evidence in the literature support the use of serum TSH concentration as the single test to detect abnormal thyroid function [11], but we want to emphasize that a more complete approach would be able to better define the status of the thyroid axis with important long-term consequence also in the era of cost effective medicine, since a more accurate diagnosis would avoid further expenses.

REFERENCES

- 1 American College of Physician. Clinical guideline, part 1. Screening for thyroid disease. *Ann Intern Med* 1998; **129**:141–3.
- 2 Helfand M, Redfern CC. Clinical guideline, part 2. Screening for thyroid disease: an update. *Ann Intern Med* 1998; **129**:144–58.
- 3 Stott DJ, McLellan AR, Finlayson J, Chu P, Alexander WD. Elderly patients with suppressed serum TSH but normal free thyroid hormone levels usually have mild thyroid overactivity and are at increased risk of developing overt hyperthyroidism. *Q J Med* 1991; **78**:77–84.
- 4 Staub JJ, Althaus BU, Engler H, Ryff AS, Trabucco P, Marquardt K et al. Spectrum of subclinical and overt hypothyroidism: effect on thyrotropin, prolactin, and thyroid reserve, and metabolic impact on peripheral target tissues. *Am J Med* 1992; **92**:631–41.
- 5 Fazio S, Biondi B, Carella C, Sabatini D, Cittadini A, Ponza N et al. Diastolic dysfunction in patients on thyroid-stimulating hormone suppressive therapy with levothyroxine: beneficial effect of beta-blockade. *J Clin Endocrinol Metab* 1995; **80**:2222–6.
- 6 Zulewski H, Muller B, Exer P, Miserez AP, Staub JJ. Estimation of tissue hypothyroidism by a new clinical score: evaluation of patients with various grades of hypothyroidism and controls. *J Clin Endocrinol Metab* 1997; **82**:771–776.
- 7 Biondi B, Fazio S, Palmieri EA, Carella C, Ponza N, Cittadini A et al. Left ventricular diastolic dysfunction in patients with subclinical hypothyroidism. *J Clin Endocrinol Metab* 1999; **84**:2064–7.
- 8 Auer J, Scheibner P, Mische T, Langsteger W, Eber O, Eber B. Subclinical hyperthyroidism as a risk factor for atrial fibrillation. *Am Heart J* 2001; **142**:838–42.
- 9 Cooper DS. Subclinical hypothyroidism. *N Engl J Med* 2001; **345**:260–5.
- 10 Toft AD. Subclinical hyperthyroidism. *N Engl J Med* 2001; **345**:512–6.
- 11 Roti E, Gardini E, Nagotti MG, Pilla S, Minelli R, Salvi M et al. Are thyroid function tests too frequently and inappropriately requested? *J Endocrinol Invest* 1999; **22**:184–190.