Thyroid Function and Prevalence of Anti-Thyroperoxidase (TPO) and Anti-Thyroglobulin (Tg) Antibodies in Outpatients Hospital Setting in an Area with Sufficient Iodine Intake: Influences of Age and Sex

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Abstract- In order to examine the prevalence of thyroid disease in a hospital outpatient setting, in an area of sufficient iodine intake, serum levels of TSH, T4, T3, anti-Tg and anti-TPO antibodies were examined in 909 individuals with an age range of 12.4 to 88.5 years, participating in a checkup outpatient setting. The study was conducted in Henry Dynant Hospital located in the metropolitan area of Athens, Greece, during a 2 year period. Hormonal parameters were determined by chemiluminescence immunoassay. Overt thyrotoxicosis was found in 4.95% of the total population and subclinical thyrotoxicosis in 5.5%. Overt hypothyroidism was found in 1.43% and subclinical hypothyroidism in 4.51%. In male population, overt thyrotoxicosis was found in 4.4 % and subclinical thyrotoxicosis was also found in 4.4%. On the other hand, overt hypothyroidism was found in 1.4% and subclinical hypothyroidism was found in 3.7% in males. In female population, overt thyrotoxicosis was found in 5.2% whereas subclinical thyrotoxicosis was found in 6.0%. Overt hypothyroidism was found in 1.5% and subclinical hypothyroidism was found in 4,9% in females. Positive anti-TPO antibodies were detected more often (30.4%) than anti-Tg (15.4%) in the tested population. The positivity in both anti-TPO and anti-Tg antibodies was correlated with abnormally high TSH concentrations after the age of 50 years, especially in female population. In conclusion distinct profile of thyroid hormonal parameters was observed in inhabitants in the metropolitan area of Athens, with overt thyrotoxicosis strikingly overcome overt hypothyroidism while subclinical forms of each dysfunction also exhibit analogous results.

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Introduction

Overt abnormalities in thyroid function are common endocrine disorders affecting 5–10% of individuals over a lifespan (1). Clinical symptoms and signs are often nonspecific, and the diagnosis and monitoring of therapy depends crucially on measurements of thyroid hormones and TSH in blood (2,3).

Minor abnormalities in thyroid function with subclinical hypothyroidism or hyperthyroidism are even more common (4,5). Both subclinical hypothyroidism and hyperthyroidism are associated with an increase in risk of disease (4-6) as well as deviations in biochemical and physiologic measures that are persistently abnormal in patients with overt thyroid disease (6-9).

Nevertheless, it is still debated to what extent subclinical thyroid disease should be treated (6,10-12). Moreover, interpretation of data on the prevalence of thyroid disease in a certain area must take into account several important parameters such as iodine intake, sex and age.

In order to evaluate the prevalence of thyroid disease and dysfunction including thyroid autoimmunity in an area with sufficient iodine intake, we performed a cross sectional study collecting data from the outpatient hospital setting in the metropolitan area of Athens, Greece in a two-year surveillance study. Moreover, we

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investigated whether thyroid function in this area was influenced by age, sex and the presence of anti-thyroperoxidase (TPO) and anti-thyroglobulin (Tg) antibodies.

Materials and Methods

The study consisted of 909 individuals, 615 (67.7%) of whom were females and 294 (32.3%) were males. The mean (SD) of age was 48.0 (19.9) ranging from 12.4 to 88.5 years. All participants were inhabitants of the city of Athens. Men were slightly older compared to women [mean (SD) age: 49.4 (16.6) vs. 47.7 (17.0) respectively] but this difference was not statistically significant (P=0.088).

Potential participants were excluded only if: 1) they were currently receiving T4 (or other thyroid hormone preparation) or antithyroid therapy; 2) during the previous 12 months, they had pregnancy, thyroid surgery, radioiodine therapy, or use of antithyroid drugs; 3) their family doctor judged that contact was inappropriate (e.g. recent bereavement,); or 4) they were unable to provide informed consent. Moreover, information was collected on age, sex, although there was no examination of the neck for goiter or thyroid size. According to the study design none of the participants were smokers, and had previously any medical record concerning thyroid, liver or kidney pathology and/or receiving any of the following medication list that is known to influence thyroid hormonal parameters: glucocorticoids, lithium, amiodarone, γ-interferon, contraceptives, dopamine agonists, β-blockers or metformine.

T3 was measured with a chemiluminescence immunometric assay (Siemens Medical Solutions Diagnostics) with a sensitivity 0.1 ng/ml, reference range of 0.1-8 ng/ml (0.15-12.3 nM) and normal range of 0.7-1.8 ng/ml.

T4 was measured with a chemiluminescence immunometric assay (Siemens Medical Solutions Diagnostics) with a sensitivity of 0.3 μg/dl, reference range of 0.3-30 μg/dl (3.9-387 nM) and normal range of 4.5-12.00 μg/dl.

TSH (TSH-3) was determined using immunochemiluminometric technology and a third-generation assay (Siemens Medical Solutions Diagnostics, USA) with a sensitivity 0.004 μIU/ml (mIU/l), reference range of 0.004-150 μIU/ml (mIU/l) and normal range of 0.4-4.5 μIU/ml.

Anti-TPO (aTPO) and anti-Tg (aTg): aTPO and aTg were measured using thyroid autoantibody immunological test system for ADVIA-CTR by Siemens Medical Solutions Diagnostics, USA, with a normal range in humans for aTPO: <60 U/ml, and for aTg: <70 U/ml.

Data and statistical analysis

Exploratory analysis and description of the characteristics of the study population was performed using two-way and three-way tables along with graphs and tables of cross-sectional medians and interquartile ranges of hormone levels by sex and age. Simple two-samples comparisons of hormone levels were performed using non-parametric tests (Mann-Whitney U-test). TSH levels have been analyzed through multivariate linear regression models after a log transformation due to the skewness of their distribution in the original scale. Exploratory analyses regarding the relations between TSH levels and factors such as age, sex and positivity in two antibodies was performed graphically using scatter plots and superimposed lowest smoothing curves. The final model used included linear and quadratic terms for age and their interactions with sex and antibodies' positivity.

For these analyses, hyperthyroidism was defined as 1) clinically significant or overt if TSH <0.1 mIU/l and T4 =154,8 nM; as 2) subclinical or mild when TSH was in the range of 0.1 to 0.4 mIU/l and T4 was in the range of <154,8 nM; and 3) euthyroid (serum TSH 0.4-4.5 mIU/l). On the other hand, hypothyroidism was defined as 1) clinically significant or overt if TSH >10 mIU/l and T4 <58.05 nM (4.5 μg/dl) and as 2) subclinical or mild when TSH was in the range of 4.5 to 10 mIU/l and T4 was in the range of 58.05 nM (4.5 μg/dl).

Because total (T4) and not free (FT4) thyroxine levels were used in this study, some total T4 concentrations may have been altered because of distorted thyroid hormone binding proteins in patients who were receiving certain concomitant medications not excluded from this study such as phenothiazines, phenytoin, or aspirin. For that reason, we categorized hyperthyroid states according to TSH levels alone, assuming that virtually all hyperthyroid patients have undetectable serum TSH levels. Accordingly, the population of patients with subclinical hypothyroidism may be, as above, overestimated.

Results

In table 1, we show the age and sex distribution of the tested population. According to our results, overt thyrotoxicosis was found in 4.95% and subclinical

thyrotoxicosis in 5.50% of the total population. Overt hypothyroidism was found in 1.43% and subclinical hypothyroidism in 4.51%. In the male population, overt thyrotoxicosis was found in 4.4% and subclinical thyrotoxicosis was also found in 4.4%. On the other hand, overt hypothyroidism was found in 1.4% and subclinical hypothyroidism was found in 3.7% in males. In the female population, overt thyrotoxicosis was found in 5.2% whereas subclinical thyrotoxicosis was found in 6.0%. Overt hypothyroidism was found in 1.5% and subclinical hypothyroidism was found in 4.9% in females.

Table 1. Sex distribution by age group.

	Gender		
Age	**Female**	**Male**	**Total**
	N (%)		N (%)
12-19	23	9	32
	71.88	28.13	100.00
20-29	71	27	98
	72.45	27.55	100.00
30-39	149	57	206
	72.33	27.67	100.00
40-49	106	64	170
	62.35	37.65	100.00
50-59	125	53	178
	70.22	29.78	100.00
60-69	65	44	109
	59.63	40.37	100.00
70-79	55	30	85
	64.71	35.29	100.00
80+	21	10	31
	67.74	32.26	100.00
Total	615	294	909
	67.66	32.34	100.00

T3 values

In the population tested, median T3 levels showed a tendency to decreased values after the age of 60 years especially in the male group (P=0.002).

The highest median values of T3 levels were reported in both sex groups at the age of 12-19 years with normal TSH levels (Table 2).

T4 values

According to our results, in the male population older than 50 years median T4 levels decreased with age but the rate of decline was not statistically significant (estimated median loss per 10 years of age: 0.19 units; p=0.140). The lowest median T4 value was found in male participants older than 80 years (Table 3). In contrast, in female participants T4 levels showed an increased tendency with age and the highest value of median T4 was found after the age of 70 years (estimated median gain per 10 years of age: 0.10 units; P=0.030).

TSH values

TSH levels seem to decline with age and this trend appears more pronounced among men aged between 30 and 70 years (Table 4). This difference in the rate of decline results in higher TSH levels for women aged between 40 and 70 years. In Table 5, the stratification of TSH levels in distinct range according to age and sex is presented. Results are provided as absolute numbers and relative proportions.

aTPO and aTg values

According to our results, a common declining trend in both aTPO (Table 6) and aTg (Table 7) positivity was observed until the age of 50 years but older subjects being positive in both aTG and aTPO showed an increasing tendency of TSH levels.

Table 2. Median (IQR) T3 levels by sex, age group and overall. *P*-values refer to comparisons between men and women of the same age group.

Age	**Female**	**Male**	***P*-value**
12-19	1.61 (1.19, 1.79)	1.58 (1.48, 1.91)	0.187
20-29	1.38 (1.21, 1.67)	1.40 (1.14, 1.50)	0.551
30-39	1.36 (1.18, 1.56)	1.35 (1.19, 1.53)	0.590
40-49	1.32 (1.16, 1.57)	1.38 (1.14, 1.57)	0.728
50-59	1.34 (1.18, 1.55)	1.33 (1.14, 1.58)	0.758
60-69	1.37 (1.13, 1.50)	1.31 (1.19, 1.47)	0.706
70-79	1.31 (1.06, 1.50)	1.18 (0.93, 1.39)	0.221
80+	1.16 (0.94, 1.47)	0.95 (0.83, 1.22)	0.300
Overall	1.35 (1.15, 1.56)	1.34 (1.14, 1.54)	0.216

Table 3. Median (IQR) T4 levels by sex and age group.

Age	Female	Male	*P*-value *
12-19	8.10 (7.00,8.80)	8.30 (7.20,8.70)	0.999
20-29	7.90 (7.10, 9.30)	8.40 (7.30, 8.90)	0.440
30-39	8.30 (7.40, 9.30)	8.00 (6.90, 8.80)	0.044
40-49	8.40 (7.50, 9.40)	8.55 (7.55, 9.65)	0.881
50-59	8.50 (7.30, 9.70)	7.80 (6.90, 9.20)	0.128
60-69	8.40 (7.20, 9.80)	8.00 (6.85, 9.40)	0.174
70-79	8.60 (7.00, 9.80)	7.80 (6.80, 9.10)	0.451
80+	8.60 (7.80, 9.80)	7.45 (6.90, 8.20)	0.083
Overall	8.40 (7.30, 9.50)	8.10 (7.00, 9.10)	0.016

* *P*-values refer to comparisons between men and women of the same age group

Table 4. Median (IQR) TSH levels by sex and age group. *P*-values refer to comparisons between men and women of the same age group.

Age	Female	Male	*P*-value *
12-19	2.86 (1.46, 3.83)	1.52 (0.99, 2.15)	0.022
20-29	1.75 (1.09, 2.41)	1.96 (1.35, 3.05)	0.342
30-39	1.70 (0.91, 2.67)	1.53 (0.88, 2.47)	0.965
40-49	1.61 (0.95, 2.44)	1.23 (0.74, 1.80)	0.034
50-59	1.37 (0.93, 2.88)	1.23 (0.76, 1.58)	0.046
60-69	1.57 (1.13, 2.38)	1.17 (0.70, 2.02)	0.063
70-79	1.34 (0.50, 2.86)	1.07 (0.64, 1.49)	0.372
80+	1.13 (0.26, 1.75)	1.04 (0.79, 1.37)	0.767
Overall	1.57 (0.94, 2.59)	1.29 (0.82, 2.00)	0.002

*P-values refer to comparisons between men and women of the same age group

Table 5. TSH levels categorized by age group and sex. Figures in each cell are counts and proportions.

	Females						Males					
Age	**<0.1**	**0.1-0.4**	**0.4-2.5**	**2.5-4.5**	**4.5-10**	**10+**	**<0.1**	**0.1-0.4**	**0.4-2.5**	**2.5-4.5**	**4.5-10**	**10+**
12-19			8	12	3				7	2		
			34.8	52.2	13.0				77.8	22.2		
20-29	1	1	53	12	4		2		16	6	2	1
	1.4	1.4	74.6	16.9	5.6		7.4		59.3	22.2	7.4	3.7
30-39	11	4	90	35	8	1		2	41	12	1	1
	7.4	2.7	60.4	23.5	5.4	0.7		3.5	71.9	21.1	1.8	1.8
40-49	5	8	70	17	4	2	1	4	53	4	2	
	4.7	7.5	66.0	16.0	3.8	1.9	1.6	6.3	82.8	6.3	3.1	
50-59	3	11	78	25	6	2	4	2	42	3	2	
	2.4	8.8	62.4	20.0	4.8	1.6	7.5	3.8	79.2	5.7	3.8	
60-69	3	5	43	11	1	2	5	2	32	2	1	2
	4.6	7.7	66.2	16.9	1.5	3.1	11.4	4.5	72.7	4.5	2.3	4.5
70-79	6	5	29	11	2	2	1	3	22	3	1	
	10.9	9.1	52.7	20.0	3.6	3.6	3.3	10.0	73.3	10.0	3.3	
80+	3	3	13		2				8		2	
	14.3	14.3	61.9		9.5				80.0		20.0	
TOTAL	32	37	384	123	30	9	13	13	221	32	11	4
	5.2	6.0	62.4	20.0	4.9	1.5	4.4	4.4	75.2	10.9	3.7	1.4

Table 6. Anti-TPO positivity by age and sex.

	Gender and Anti-TPO					
	Female		Male		Total	
Age	-(<60	)+(>60)	-(<60)	+(>60)	-(<60)	+(>60)
12-19	21	2	9		30	2
	91.3	8.7	100.0		93.8	6.3
20-29	50	21	16	11	66	32
	70.4	29.6	59.3	40.7	67.3	32.7
30-39	93	56	47	10	140	66
	62.4	37.6	82.5	17.5	68.0	32.0
40-49	70	36	53	11	123	47
	66.0	34.0	82.8	17.2	72.4	27.6
50-59	76	49	38	15	114	64
	60.8	39.2	71.7	28.3	64.0	36.0
60-69	46	19	32	12	78	31
	70.8	29.2	72.7	27.3	71.6	28.4
70-79	34	21	24	6	58	27
	61.8	38.2	80.0	20.0	68.2	31.8
80+	14	7	10		24	7
	66.7	33.3	100.0		77.4	22.6
Total	404	211	229	65	633	276
	65.7	34.3	77.9	22.1	69.6	30.4

Numbers in each cell are counts and proportions.

A predominance of the female gender concerning both the aTPO and aTg positivity is detected in all age groups. However, it should be noted that the majority of the study population was negative for both aTG and aTPO and thus the absolute number of individuals being positive in both antibodies was relative small.

Table 7. Anti-TG positivity by age group and sex.

	Gender and Anti-TPO					
	Female		Male		Total	
Age	-(<70)	+(>70)	-(<70)	+(>70)	-(<70)	+(>70)
12-19	21	2	9		30	2
	91.3	8.7	100.0		93.8	6.3
20-29	58	13	19	8	77	21
	81.7	18.3	70.4	29.6	78.6	21.4
30-39	122	27	54	3	176	30
	81.9	18.1	94.7	5.3	85.4	14.6
40-49	84	22	58	6	142	28
	79.2	20.8	90.6	9.4	83.5	16.5
50-59	101	24	49	4	150	28
	80.8	19.2	92.5	7.5	84.3	15.7
60-69	53	12	38	6	91	18
	81.5	18.5	86.4	13.6	83.5	16.5
70-79	46	9	28	2	74	11
	83.6	16.4	93.3	6.7	87.1	12.9
80+	20	1	9	1	29	2
	95.2	4.8	90.0	10.0	93.5	6.5
Total	505	110	264	30	769	140
	82.1	17.9	89.8	10.2	84.6	15.4

Numbers in each cell are counts and proportion

Table 8. Distribution of T4 and TSH according to T3 levels.

	T3 Low (<0.8) N (%)	Normal (0.8-2) N (%)	High (>2) N (%)	Overall N (%)
T4				
Low (<4.5)	11 (45.8)	4 (0.5)	0 (0.0)	15 (1.7)
Normal (4.5-12)	13 (54.2)	834 (97.1)	9 (34.6)	856 (94.2)
High (>12)	0 (0.0)	21 (2.4)	17 (65.4)	38 (4.2)
TSH				
Low (<.4)	9 (37.5)	73 (8.5)	13 (50.0)	95 (10.5)
Normal (.4-4.5)	9 (37.5)	739 (86.0)	12 (46.2)	760 (83.6)
High (>4.5)	6 (25.0)	47 (5.5)	1 (3.8)	54 (5.9)
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
T4	4.8 (4.0, 6.2)	8.3 (7.3, 9.3)	12.9 (11.0, 20.7)	8.3 (7.2, 9.4)
TSH	1.2 (0.1, 4.0)	1.5 (0.9, 2.4)	0.3 (0.0, 1.5)	1.5 (0.9, 2.4)

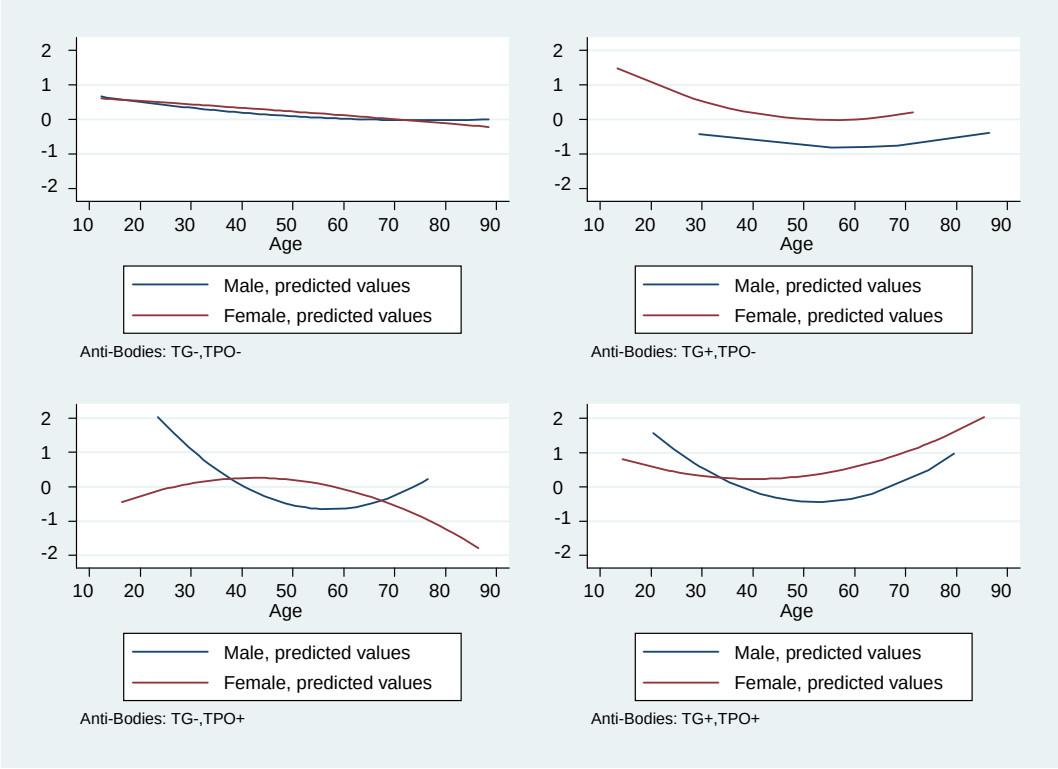

Figure 1. Predicted TSH levels (log-scale): results from a multivariate linear regression models including linear and quadratic age terms along with their interactions with sex and antibodies' positivity.

Correlations between T3, T4 and TSH

T3 and T4 showed a significant (p<0.001) moderate positive correlation (Spearman's rho=0.463). On the contrary, T3 and TSH were practically independent (Spearman's rho=0.035, *P*=0.295) whereas T4 and TSH had a weak but significant negative correlation (Spearman's rho=-0.205, *P*<0.001). These findings were practically consistent among both men and women. The positive correlation between T3 and T4 seemed to become progressively stronger at older individuals (e.g. Spearman's rho=0.437 and 0.601 for individuals aged 20-29 and 80+ years respectively). The weak negative correlation between T4 and TSH did not show any particular trend with age (Table 8).

Interrelationships of analytes

In figure 1, we show the predicted TSH levels (log-scale) estimated from a multivariate linear regression

models including linear and quadratic age terms along with their interactions with sex and antibodies' positivity. All interactions included in the model were statistically significant ($P<0.05$). Predicted lines seem consistent with the trends seen in the observed data through the LOWESS smoother. It should be noted that the expected variability of the TSH levels calculated by this model (R2) was only 7%, indicating that age, sex and positivity of aTg and aTPO antibodies are not enough to predict TSH values accurately and most probably many other factors contribute to the observed variability. According to the regression models, the following conclusions can be detected:

a) TSH levels of both men and women with both aTG and aTPO negative seem to decline with age with a common rate resulting to comparable levels across a wide range of ages.
b) Women with at least one of the two antibodies positive seem to have higher TSH levels compared to men of the same age for a range of ages between 40 and 70 years.
c) TSH levels of individuals with both antibodies positive seem to increase after the age of approximately 50 years.

Discussion

Thyroid dysfunction is common (13-16). Interpretation of data on the prevalence of thyroid disease in a certain area must take into account several important parameters such as iodine intake, sex and age (17). For instance, hypothyroidism is far more common among elderly women than among other groups, particularly in the presence of thyroid autoantibodies (13-15).

With the awareness that subclinical and clinical forms of hyperthyroidism and hypothyroidism are emerging as potential contributors to morbidity from osteoporosis, hyperlipidemia, hypercholesterolemia, hyperhomocysteinemia and cardiovascular and neuropsychiatric disease, especially in the older population (18-22), the results of the present study estimate the current thyroid status and also provide reference data for TSH, T4, aTg, and aTPO in the capital area of Greece.

Our study can be compared with several other population-based studies. The Whickham survey, performed in the northeastern part of England, an iodine-replete area, at a time when no sensitive TSH assays were available, found thyrotoxicosis in 1.6%, overt hypothyroidism in 1.1%, and a TSH concentration 6 mIU/l in 5.0% of participants (15). A large study performed in Colorado with a TSH cutoff concentration of 5.1 mIU/l found twice as much subclinical hypothyroidism (9.0%) (23). A Danish survey in an area with borderline iodine deficiency found thyrotoxicosis in 2.0% and hypothyroidism in 1.4% of the participants, with subclinical hypothyroidism in only 0.6% of participants (24). German researchers recently concluded that reference intervals of thyroid function tests in a formerly iodine-deficient region are distinct from the reference intervals that were established in areas with iodine sufficiency (25).

In the third National Health and Nutrition Examination Survey (NHANES III), a recent large population-wide survey in the United States, hypothyroidism was found in 4.6% (0.3% overt and 4.3% subclinical) and hyperthyroidism in 1.3% (0.5% overt and 0.7% subclinical) of the total population (14). In our study, although the prevalence of hypothyroidism was similar to the above presented studies, namely in 5.9% of the total population (1.43% overt and 4.51% subclinical), the prevalence of hyperthyroidism was higher (10.45% of the total population, 4.95% overt and 5.50 % subclinical). However, we have to mention that the above findings should be interpreted taking also into account the cross-sectional nature of the current study. In particular, a prospective longitudinal study would be far more informative regarding for instance the evolution of TSH levels as an individual ages and factors affecting.

Thyroid abnormalities in populations with low iodine intake and in those with high iodine intake develop in opposite directions: more goiter and thyroid hyperfunction are seen when iodine intake is relatively low, whereas more hypothyroidism is seen when iodine intake is relatively high. This trend was demonstrated very elegantly in a study by Laurberg *et al.* (17), that compared elderly persons in Iceland (an area with a high iodine intake) with elderly persons in Jutland (an area with a low iodine intake). In Iceland, hypothyroidism was common, whereas in Jutland, hyperthyroidism was common and hypothyroidism was rare.

Although, the pattern of thyroid dysfunction seen in our study is compatible with a marginally sufficient iodine intake in our area, other explanations might also occur according to recent documentations. In Greece, iodine supplementation of salt and water was initiated after field studies in the late 1960s (26-30). It has been estimated, in present time, that dietary iodine intake in Athens has almost reached the optimum level of about 200 mg iodine/day (31). Moreover, increased iodine intake has been implicated in fueling the thyroid

autoimmune process in both humans (32) and experimental animals (33,34), resulting in the development of either Hashimoto's thyroiditis or Graves' disease, that might explain our recent findings. Indeed, recent clinical data clearly suggests that the elimination of iodine deficiency has been accompanied by an increase in the prevalence of autoimmune thyroiditis in many areas of the world as well as in Greece (35-37).

Interestingly, in our study a progressive decline of TSH values within the normal limits was observed in relation to aging, although individuals with both positive antithyroid antibodies (aTPO, aTg) exhibited an increase in TSH distribution with aging. Several clinical studies have shown that due to an established increase in prevalence of antithyroid antibody with age (14,38) the parallel increase in serum TSH distribution in these cases could be attributed to an increase in prevalence of acquired autoimmune thyroid disease. Nevertheless, recent reports indicate that serum TSH concentrations and distribution gradually increase with age (14), suggesting either a decline in thyroid function or a reset in the TSH set point, which may occur with aging. Moreover, Atzmon *et al.* (39) findings support other reports demonstrating elevated serum TSH concentrations in extreme longevity (40,41). Interestingly, in line with our findings, other studies also show a decrease in serum TSH levels in centenarians (42,43). For that reason, Atzmon *et al.* (39) suggest that their data should be interpreted cautiously due to several reasons such as numbers of subjects, variable iodine deficiency, different genetic backgrounds and their findings should not, therefore, be extrapolated to populations outside of North America. Additionally, Boucai *et al.* (44) in an attempt to determine whether race and age influence the distribution of TSH and free T4 (fT4) in a cross-sectional study of an urban outpatient medical practice clearly emphasize the need to develop TSH reference limits that consider both race and age in order to minimize misclassification of patients with thyroid disease.

Concerning the presence of aTPO in subclinical hypothyroidism is known to be associated with a higher risk of developing overt hypothyroidism later in life (45). Nevertheless, it has been noted that the use of aTPO screening, does not lead to the detection of all individuals with mild thyroid failure attributable to autoimmunity, as can be concluded from the substantial percentage of those with hypothyroidism who had negative aTPO in clinical studies (46). In our study, both aTPO and aTg positivity was increased in female population compare to males and correlated with high TSH concentrations after the age of 50 years, although due to the variability of the predicted TSH values in respect to age, sex and positivity of aTPO and aTg antibodies in multivariate analysis raises questions concerning the validation of aTPO and aTg.

In line with previous findings (47), our data indicate that the distinction between subclinical and overt thyroid disease is somewhat arbitrary because it depends to a considerable extent on the position of the patient's normal set point for T3 and T4 within the laboratory reference range. The pituitary gland is sensitive to minor changes in serum T3 and T4, and serum TSH responds heavily to such changes. When thyroid function is abnormal, the association between serum TSH and both T3 and T4 is log linear (48,49). This amplified response of serum TSH to changes in serum T3 and T4 may cause serum TSH to leave the population based reference range when serum T3 and T4 are outside the individual reference range, even when they are still within the population-based reference range. This is labeled subclinical thyroid disease. The view that individuals with subclinical thyroid disease have abnormal thyroid function is supported by increasing amounts of data on the biological importance of subclinical thyroid disease for a number of organs. In conclusion, inhabitants in the metropolitan area of Athens, known to be an area with sufficient iodine intake, show a distinct profile of thyroid hormonal parameters with overt thyrotoxicosis strikingly overcome overt hypothyroidism while subclinical forms of each dysfunction exhibit analogous results. Interestingly, although serum T3 and T4 gradually decreased with age, serum TSH showed a tendency to lower normal limits.

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