CLINICAL STUDY

Lower early morning plasma cortisol levels are associated with thyroid autoimmunity in the elderly

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Abstract

Objectives: Thyroid autoimmunity decreases in the very old. We investigated whether glucocorticoid (GC) activity, which increases in old age, is involved in this process.
Subjects and methods: A total of 321 ambulatory subjects (age 51–95 years, median 71, 207 female) were studied. Thyroid function tests, cortisol, glucose, insulin and biochemical parameters were measured. A modified overnight dexamethasone suppression test (0.25 mg) was performed as an index of GC sensitivity.
Results: Forty subjects had positive anti-thyroid peroxidase antibodies and 36 had positive anti-thyroglobulin antibodies, while 57 had either one or the other or both thyroid autoantibodies (ThAbs) positive. Mean basal cortisol levels were significantly lower in the ThAbs (+) groups (320 ± 125 vs 378 ± 128 nmol/l, $P = 0.002$). Triiodothyronine, free thyroxine, post-dexamethasone cortisol levels, C-reactive protein, homeostasis model assessment-insulin-resistance-index and body mass index did not differ between these two groups. Mean age of ThAbs (+) subjects was lower compared to the ThAbs (−) group (67.38 ± 7.38 vs 71.64 ± 8.57 years, $P = 0.001$).
Conclusions: Reduced GC activity is associated with an increased prevalence of ThAbs positivity in older ambulatory subjects. Subjects without ThAbs in this population sample are relatively older. It is not known whether this is related to increasing GC activity with age.

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Introduction

Immunosenescence is a well-characterized phenomenon accompanying ageing and represents a malfunction of the immune system leading to both increased infection susceptibility and increased frequency of certain kinds of autoimmune disorders in older age (1, 2). Several mechanisms have been postulated (3, 4) including neuroendocrine changes (5). Of particular interest is the role of the stress and hypothalamus–pituitary–adrenal (HPA) axis activity in this process (6). Ageing is accompanied with activation of the HPA axis and increased levels of ACTH and cortisol (7–9). Ageing has also been associated with changes in the immune system sharing many common characteristics with the alterations in immune function seen in chronic stress (10) and/or glucocorticoid (GC) treatment (11).

Autoimmune thyroiditis is a common autoimmune disorder and a typical example of an organ-specific autoimmune disorder. Various studies have assessed the prevalence of thyroid autoimmunity in the normal population. Both female gender and increasing age have been associated with a higher frequency of thyroid antibody positivity (12, 13). However, a few studies concerning healthy centenarians and selected groups of ambulatory elderly people have shown decreasing prevalence of thyroid autoimmunity with age, which then approaches that of younger age groups (14, 15).

The aim of this study was to assess thyroid autoimmunity in a population sample consisting of apparently healthy elderly subjects and to examine how the ageing process affects this phenomenon; furthermore, possible associations of thyroid autoimmunity with the HPA axis activity and with various metabolic parameters were examined.

Subjects and methods

We studied a final number of 321 community-dwelling elderly individuals (207 women, 114 men, aged 51–95 years) who were all permanent residents of a rural area in Peloponnese (Southern Greece). All subjects were recruited through an announcement in the local Recreation Centre for the elderly that offered a preventive screening for thyroid function and general health evaluation. Nearly all the elderly ambulatory subjects of this area regularly visit this recreation

centre, and the majority were female. In total 85% of the regular attendees presented for evaluation. A detailed medical history (including administered medication) was recorded, and a physical examination was performed (including thyroid gland palpation, arterial blood pressure measurement, weight, height, waist and hip circumference measurements). All the subjects included in this study were born in this area and have lived in this area practically all their lives.

Thyroid function tests (free thyroxine (FT_4), TSH, triiodothyronine (T_3), anti-thyroglobulin (TG) and anti-thyroid peroxidase (TPO) autoantibodies), basal cortisol, glucose, insulin, lipid levels and biochemical profile were determined between 0730 and 0900 h after an overnight fast. An ultra-low dose (0.25 mg) dexamethasone suppression test (DST) was performed in 232 of the participants in the study, as it was initiated from a certain point onwards, as previously described (16). Subjects ingested 0.25 mg dexamethasone at 2300 h, and a venous blood sample for cortisol, insulin and glucose levels measurement was obtained the following morning (after overnight fast) between 0730 and 0900 h. The level of cortisol suppression, defined as delta-cortisol, was calculated by subtracting post-dexamethasone suppression cortisol levels from basal cortisol levels. This test has been used before as an indirect index of hypothalamus–pituitary sensitivity to GC (17).

From the original group who presented for evaluation, subjects with a history of coronary artery disease ($n = 31$), cerebrovascular disease ($n = 11$) and cancer ($n = 6$) were excluded from the study. Furthermore, two individuals who were diagnosed during the study with previously unknown hypothyroidism (TSH levels > 10 mIU/l), three who had abnormal liver function tests and eleven who had creatinine levels above 1.3 mg/dl were also excluded from the final statistical analysis, leaving a total of 321 subjects that represented the final cohort of the initial analysis. All women were postmenopausal and did not receive hormone replacement therapy. A total of 23 subjects were on L-T_4 treatment and 25 had a past diagnosis of thyroid disease. A significant proportion ($n = 63$) of the studied individuals had diabetes mellitus (defined as either a history of diabetes mellitus, or a fasting glucose level ≥ 7.0 mmol/l). Because cortisol levels may be influenced by the diabetic status and thyroid antibodies presence may be affected by a history of thyroid disease or thyroid function abnormalities, a separate analysis was performed after exclusion of the subjects having a history of diabetes mellitus or a fasting glucose > 6.1 mmol/l and those with known thyroid disease. The 'apparently normal' group consisted of 224 subjects (14 subjects had both disorders).

The study was approved by the institutional ethics committee, and all subjects gave their informed consent.

Serum insulin, TSH, FT_4, T_3, serum thyroid autoantibodies (ThAbs; anti-TPO and anti-TG) and cortisol were measured using chemiluminescent immuno-metric assays with the DPC Immulite 2000 (Siemens, Llanberis, Gwynedd, UK). Reference range was TSH 0.36–4 mIU/l, FT_4 9–26 pmol/l, T_3 1.1–2.9 nmol/l, anti-TPO < 30 IU/ml and anti-TG < 40 IU/ml. Positive ThAbs were considered if either anti-TPO or anti-TG or both autoantibodies were above the upper normal limit of their normal reference range. The population was then divided into two groups according to their thyroid antibody positivity: ThAbs $(+)$ and ThAbs $(-)$.

The levels of glucose, total cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, C-reactive protein (CRP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen and creatinine were measured using an automated analyzer, Architect c8000, Abbott. Basal insulin-resistance (IR) index (homeostasis model assessment-IR-index) was calculated according to the formula: $IR = FI \times G/22.5$, where FI, fasting insulin (μIU/ml) and G, fasting glucose (mmol/l).

Statistical analysis

Statistical analysis was performed using the SPSS statistical package version 11 (SPSS Inc., Chicago, IL, USA). All descriptive data are presented as mean ± S.D. Student's *t*-test was used to compare mean values between groups where the distribution was normal, and Mann–Whitney non-parametric test was used when the distribution of values was not normal (i.e. whenever Levene's test for homogeneity of variance was significant). The mean baseline and post-DST cortisol levels were compared using the two-sample Wilcoxon rank-sum test, while the association between them was assessed using a stepwise linear regression model adjusted for age, body mass index (BMI) and liver function tests. The χ^2 test was used to analyze the differences in ThAbs frequency in various subgroups of the population. Binary logistic regression analysis was performed to analyze the predictive value of various parameters for the presence of ThAbs (using an adjusted stepwise forward likelihood ratio model). The Hosmer and Lemeshow test was checked for non-significance at each step to ensure that the model adequately fitted the data. The odds ratio (OR) of each variable for the positivity of ThAbs and the respective confidence intervals (CI) were calculated. The significance level (*P* value) for all of the performed analyses was defined as $P < 0.05$.

Results

The baseline characteristics of the participating subjects are shown in Table 1. The mean age of the sample population was 70.83 years (range 51–95). The mean age of the male subset (74.2 ± 8.2) of our population was significantly higher than the mean age of females

Table 1 Anthropometric and biochemical parameters in the studied population (thyroid autoantibodies (ThAbs) (+), either anti-Tg or anti-TPO or both anti-Tg and anti-TPO positive).

Male	$n=114$ (35.5%)
Female	$n=207$ (64.5%)
Mean age (years)	70.59 (± 8.8)
Body mass index (BMI; kg/m^2)	29.6 (± 5.3)
Waist-to-hip ratio (W/H)	0.74 (± 0.07)
TSH (mIU/l)	1.84 (± 1.87)
Anti-TPO (+)	$n=40$ (12.5%)
Anti-TG (+)	$n=36$ (11.2%)
ThAbs (+)	$n=57$ (17.8%)

(69.1 ± 8.2 years; $P < 0.001$). The mean BMI of the studied subjects was 29.3 ± 8.2 kg/m^2. Positive thyroid antibodies (either one or both of them) were found in 17.8% of the subjects studied. The female subjects had a significantly higher frequency (23.4%) of positive ThAbs compared to male subjects (6.4%) (Pearson $x^2 = 11\,615$, $P = 0.001$, Fisher's exact $= 0.001$).

Mean TSH was 1.84 mIU/l and did not differ between male and female subjects (Table 1). The prevalence of subclinical hypothyroidism (defined as TSH 4.5–10 mIU/l) in our sample population was 3.2%. The mean age of subjects with subclinical hypothyroidism (72.56 ± 9.6) did not differ from subjects with TSH < 4.5 mIU/l (70.65 ± 8.5 years; $P = 0.51$). Mean age and TSH levels did not differ significantly between those with and without goitre. The mean age in subjects with goitre was 71.35 ± 8.7 and in subjects without goitre was 70.95 ± 8.3 ($P = 0.76$), and the mean TSH in subjects with goitre was 1.73 ± 0.97 and in subjects without goitre was 1.81 ± 0.89, $P = 0.53$.

The differences of various parameters studied in this population according to their thyroid antibody positivity status are summarized in Table 2. The mean age of the ThAbs (+) group (67.38 ± 7.38 years) was significantly lower than the mean age of the ThAbs (−)

Table 2 Comparison of various parameters according to thyroid autoantibody positivity (mean $\pm$ s.d.).

|	ThAbs (−)	ThAbs (+)	P
Age (years)	71.64 (± 8.57)	67.38 (± 7.38)	0.001[a]
TSH (mIU/l)	1.61 (± 0.98)	2.36 (± 1.83)	0.02[b]
T_3 (nmol/l)	1.2 (± 0.24)	1.18 (± 0.24)	NS
FT_4 (pmol/l)	16.9 (± 3.1)	16.2 (± 3.9)	NS
Baseline cortisol (nmol/l)	378 (± 128)	320 (± 125)	0.002[a]
Cortisol post 0.25 mg DST (nmol/l)	168 (± 118)	143 (± 90)	NS
CRP (IU/l)	0.33 (± 0.5)	0.24 (± 0.25)	NS
Waist-to-hip ratio	0.92 (± 0.07)	0.91 (± 0.069)	NS
HOMA index	2.2 (± 1.93)	2.6 (± 3.75)	NS
Body mass index (BMI; kg/m^2)	29.4 (± 5.2)	30.5 (± 5.9)	NS

[a]Student's test.
[b]Mann–Whitney test.

group (71.64 ± 8.57 years). Baseline cortisol and TSH levels also differed significantly: the ThAbs (+) group had significantly lower mean cortisol and significantly higher mean TSH levels compared to the ThAbs (−) group (see Table 2). These results were similar when only the 'apparently normal' subjects were included. In the 'apparently normal' ThAbs (+) group, the mean age was 66.94 ± 7.2 years (compared to 71.85 ± 8.4 years in the ThAbs (−) group, $P = 0.02$, Student's test), mean cortisol levels were 313.78 ± 105.6 nmol/l (compared to 376.81 ± 123.2 nmol/l in the ThAbs (−) group, $P = 0.06$, Student's test) and TSH levels were 2.2 ± 1.2 mUI/l (compared to 1.67 ± 0.82 mUI/l in the ThAbs (−) group, $P = 0.05$, Mann–Whitney test). The results were also not different when the same analysis was performed with each of the ThAbs (anti-TPO and anti-TG) used individually as the grouping variable (data not shown). Mean cortisol levels of males (390 ± 129 nmol/l) were significantly higher than mean cortisol levels of females (353 ± 126 nmol/l; $P = 0.016$).

The mean levels of post-DST cortisol (164.2 ± 113.5 nmol/l) compared to the mean baseline cortisol levels (367.3 ± 129.3 nmol/l) were significantly lower ($P < 0.001$, two-sample Wilcoxon rank-sum test). The association of baseline cortisol with post-DST cortisol levels was studied using a stepwise multiple linear regression model adjusted for age, BMI and liver function tests (ALT and AST aminotransferases). Only baseline cortisol levels ($r = 0.322$, $P < 0.001$) and age ($r = 0.158$, $P = 0.014$) had a significant positive correlation with post-DST cortisol levels. The degree of cortisol suppression (delta-cortisol) did not differ according to thyroid antibodies presence (189.7 ± 130.7 in the ThAbs (−) group and 177.4 ± 149.9 in the ThAbs (+) group, $P = 0.580$.) Dexamethasone concentrations were not measured in this study.

The sample population was divided arbitrarily into three age intervals (each including a 15-year-range): '50–65 years', '65–80 years' and '>80 years'. The prevalence of thyroid autoantibody positivity was calculated in each of these groups, and the resulting frequencies are illustrated in Fig. 1. Totally, 34% of individuals aged 50–65 years had positive ThAbs, whereas the ThAbs frequency for the age groups '65–80 years' and '>80 years' was 18 and 4% respectively (Fig. 1). These differences remained significant when the age groups were analyzed in pairs. The '50–65 years' group compared to the '65–80 years' group had a significantly higher rate of ThAbs positivity (Pearson $x^2 = 6.73$, $P = 0.009$, Fisher's exact $= 0.012$). The '65–80 years' group compared to the '>80 years' group also had a significantly higher frequency of positive ThAbs (Pearson $x^2 = 6.53$, $P = 0.011$, Fisher's exact $= 0.012$).

The value of age and cortisol as predictors for the positivity of ThAbs was studied using logistic regression analysis. A stepwise forward likelihood ratio model
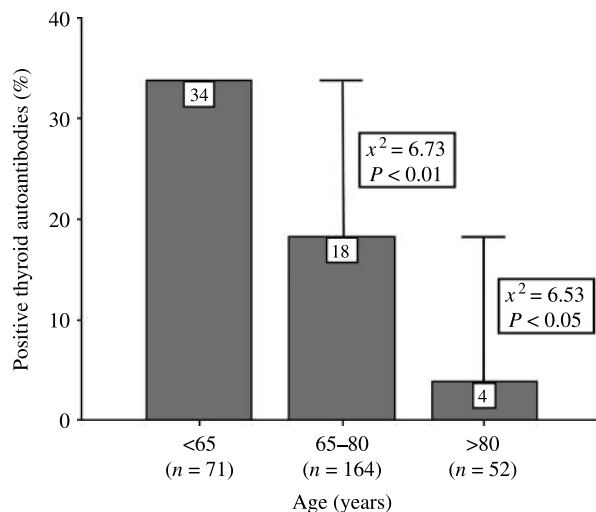
Figure 1 Prevalence of thyroid autoantibodies in the three different age groups of elderly ambulatory individuals; statistical difference in comparison with preceding group.

adjusted for gender, body weight, serum creatinine, glucose, the presence of goitre and CRP levels was used. The adjusted OR for positive ThAbs for 1-year increase in age was 0.957 (95% CI: 0.921–0.995), and the respective adjusted OR for 10 nmol/l increase in cortisol levels was 0.969 (95% CI: 0.943–0.995). The predictive value of both age and cortisol remained significant when the same analysis was conducted in the 'apparently normal' group. Similar results were obtained when subjects with TSH <0.4 were excluded (Table 3).

When 'apparently normal' female subjects were studied separately, both age and basal cortisol remained significant predictors for ThAbs positivity (OR=0.937, 95% CI: 0.892–0.984 and OR=0.967, 95% CI: 0.935–0.999 respectively). On the other hand, when

Table 3 Effect of age and baseline morning cortisol levels on the presence of positive thyroid autoantibodies (ThAbs; stepwise forward likelihood ratio model adjusted for gender, the presence of goitre, body weight, serum creatinine, glucose and C-reactive protein levels) in elderly ambulatory subjects.

	Odds ratio for ThAbs positivity	CI (95%)
All subjects		
Age[a]	0.957	0.921–0.995
Cortisol[b]	0.969	0.943–0.995
Subjects with no diabetes or known thyroid dysfunction and with TSH levels 0.4–3.6 mU/l		
Age[a]	0.932	0.888–0.978
Cortisol[b]	0.951	0.916–0.988

CI, confidence intervals
[a] One year increase in age.
[b] 10 nmol/l increase in baseline cortisol levels.

only the male subjects were studied, then cortisol levels remained a significant predictor variable (OR: 0.810, 95% CI: 0.695–0.943), while age did not (OR=1.092, 95% CI=0.910–1.311, P=0.34).

Discussion

Results from studies examining thyroid autoimmunity prevalence are not directly comparable due to the diversity in the applied biochemical methodology, the study design and different population characteristics. The overall prevalence of positive ThAbs found in this cross-sectional study (17.8%) is similar to that reported in other epidemiological studies (12, 13). The female predominance in thyroid autoimmunity, which has been found in many other studies, was also confirmed. Furthermore, we found a decrease in the prevalence of ThAbs associated with age in females. At first sight, this finding appears to be in contrast to other studies where an age-associated increase in thyroid autoimmunity has been found in females (12, 13). However, in those studies, women of all age groups were included, whereas in the current study the participating subjects were all above 50 years (the oldest was 95 years old, the median age was 71 years); the population studied by Pedersen *et al.* (12) had an age range from 18 to 65 years, while the study by Hollowell *et al.* (NHANES III) (13) covered practically all age groups. A decreased prevalence of thyroid autoimmunity in older age has been reported before in studies examining healthy centenarians and a selected population of healthy elderly people aged more than 65 years (14, 15); our results concur with the latter studies.

It has been hypothesized that the decline in thyroid autoimmunity observed in the older subgroup of elderly females may be a consequence of a selection effect (15). As the presence of positive ThAbs has been associated with increased cardiovascular risk (18, 19), one could speculate that patients with Hashimoto's thyroiditis may have a higher mortality rate at a younger age than subjects with negative ThAbs (15).

It should be pointed out that our population consisted of apparently healthy elderly subjects. Subjects with serious co-morbidities were excluded from our final analysis. It is thus possible that the age-associated decline in ThAbs is specifically found in studies including only disease-free females aged above 65 years. In studies performed in unselected populations (including hospitalized patients), the rise in thyroid antibody frequency with age may persist in the older age groups (2).

It should be noted that in the NHANES III study that also included elderly ambulatory persons (13), a steady rise in thyroid antibodies prevalence was noticed even in the 'above 80 years female disease-free' group (reaching 26.5% frequency of positive anti-TPO antibodies). Differences in iodine supply may contribute to this

difference (12), and while iodine adequacy has not been evaluated in our study, previous studies from south–western Greece, where this study was conducted, showed that this is an iodine-replete area (20, 21). Another possible explanation is differences in the prevalence of subclinical hypothyroidism. The overall frequency of thyroid dysfunction (defined as TSH > 4.5 mIU/l) in our sample was 3.8% (including those with subclinical hypothyroidism and two subjects with clinical hypothyroidism), whereas in the NHANES III trial it was 4.7%. In our study, an age-associated increase in subclinical hypothyroidism was not demonstrated, contrary to the NHANES III study results. One further factor that might be influencing the presence of ThAbs could be the presence of long-standing goitre (22). However, in our cohort, the presence of goitre did not have any influence, possibly because the age range studied was rather narrow.

One interesting mechanism that might be involved in the lower prevalence of thyroid autoimmunity in the very old could be alterations in the HPA axis that we explored in our study. We mainly used baseline early morning cortisol levels for HPA axis activity assessment. Basal morning cortisol may not always be representative of the 24-h cortisol production; however, it has been previously shown to have low intraindividual variability and to correlate well with the feedback sensitivity of the HPA axis (an association confirmed in our study) (16). Cortisol levels were lower in those with positive ThAbs, and this association was independent of age. Further analysis, according to gender, showed that this association was significant in both the male and female 'apparently normal' subsets of our population. Decreased GC activity has previously been associated with thyroid autoimmunity both in humans (23–26) and in laboratory models of autoimmune thyroid disease (27). As to the mechanisms that have been suggested to be involved in such associations, it is worth mentioning that hypercortisolaemia (due to stress or ageing) may lead to decreased hypothalamus and pituitary sensitivity to GCs, subsequent activation of the HPA axis and increased peripheral GC action ultimately leading to significant immune function changes, mainly consisting of a reduced pool of naive T-cells and a cytokine profile shift from a Th1 to a Th2 response (6). The Th2 type immune response as a result of GC action seems to have a protective role against certain types of autoimmunity (26). However, it should be noted that in our study, we simply found cortisol levels in the ThAbs positive group that were lower than the relatively 'normal' levels of the ThAbs negative group. Therefore, a protective role of increased GC activity on thyroid autoimmunity cannot be assumed based on the results of our study.

One further factor that might be involved in this process could be the effect of age on cortisol levels. A lot of studies have examined the alterations of cortisol metabolism occurring through ageing. We have found that baseline cortisol levels increase with age (28), a finding compatible with other studies (7, 9). It is not known if this age-associated increase in cortisol levels contributes to the declining thyroid autoimmunity in the elderly. In our study, both cortisol levels and age were found to be independent determinants of decreased prevalence of thyroid autoimmunity but the extent of this interaction is difficult to identify.

We also examined the possibility that sensitivity to GCs might play a role as GC negative feedback inhibition also seems to be impaired in the elderly (29, 30). In our study, we used the 0.25 mg DST as a means of assessing central feedback sensitivity to GCs (16, 17). Using this indirect index of GC sensitivity, we confirmed the decreased sensitivity of the HPA axis in the elderly, but we did not find any association with ThAbs presence. Our study may not have been powerful enough to reveal such an association. It should be noted, however, that the ultra-low dose DST is only a rough index of sensitivity to GCs, only suitable for population studies. Therefore, it is not clear whether the observations that we made have the same significance for ThAbs positivity. Another possible explanation that should be discussed is that patients with positive ThAbs may also have more frequently some form of subclinical auto-immune adrenalitis, which may account for the lower observed baseline cortisol levels. ACTH levels were not measured to assess this possibility; however, one has to note that the presence of coexisting autoimmune diseases is rather rare in patients with thyroid autoimmunity (31).

The present study has some obvious limitations, including its cross-sectional nature. The population sample studied might not be representative of the general population since it included only ambulatory elderly people and only the subjects who responded to the invitation for a health survey. It should be noted, however, that over 85% of the population participated, and that our results concerning the prevalence of autoimmune thyroiditis are consistent (even though not directly comparable) with other epidemiological studies and that the conclusions are roughly similar. The subjects examined in our study were overweight, although this did not appear to influence the association of cortisol and age with thyroid antibody positivity. It is also possible that an age-associated decline in thyroid autoimmunity in men may have not been detected due to the small number of male participants and the low prevalence of thyroid autoimmunity in males.

In conclusion, our study confirmed that the prevalence of thyroid autoimmunity decreases in the apparently healthy elderly. It further confirmed that the occurrence of thyroid autoimmunity has significant differences between males and females. The decreasing prevalence of thyroid autoimmunity in the healthy elderly appeared to be female specific. The results of our study taken together with other epidemiological

studies show a gradual rise of thyroid autoimmunity prevalence in females until approximately a decade after menopause and thereafter a gradual decline. One cannot exclude the possibility that the age-associated increase in cortisol levels may contribute to the declining thyroid autoimmunity in the elderly as we found that both cortisol levels and age were independent determinants of decreased prevalence of thyroid autoimmunity; however, the extent of this interaction cannot at present be clarified.

Declaration of interest

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