CLINICAL STUDY

Age and stress as determinants of the severity of hyperthyroidism caused by Graves' disease in newly diagnosed patients

Xander G Vos, Natalie Smit, Erik Endert¹, Jos F Brosschot², Jan G P Tijssen³ and Wilmar M Wiersinga

Department of Endocrinology and Metabolism, F5-161, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, ¹Laboratory of Endocrinology, Department of Clinical Chemistry, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands, ²Division of Clinical and Health Psychology, Department of Psychology, Leiden University, Wassenaarseweg 52, 2333 AK, Leiden, The Netherlands and ³Department of Cardiology, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ, Amsterdam, The Netherlands

(Correspondence should be addressed to X G Vos; Email: x.g.vos@amc.nl)

Abstract

Objective: The evidence that stress may provoke Graves' hyperthyroidism in genetically susceptible subjects is substantial. Whether exposure to stress is related to the severity of thyrotoxicosis has not been studied. Advancing age is associated with not only less severe Graves' hyperthyroidism but also self-reported stress. We tested the hypothesis whether advancing age is associated with less exposure to stress, resulting in a lower immunological response, and less severe Graves' hyperthyroidism.

Design: Cross-sectional multicenter study.

Patients: Two hundred and sixty-three consecutive untreated patients with a first episode of Graves' hyperthyroidism were included. The severity of Graves' hyperthyroidism was evaluated biochemically (freeT₄-index and freeT₃-index, thyrotropin-binding inhibitory immunoglobulin (TBII)) and clinically by the hyperthyroid symptom scale score (HSS score). Stress exposure was quantitated by three questionnaires.

Results: Advancing age was associated with less severe Graves' hyperthyroidism, both biochemically by lower serum freeT₃-index and freeT₄-index ($P < 0.01$), lower serum TBII ($P = 0.05$), and clinically by lower HSS scores ($P = 0.04$) and smaller goiter size ($P < 0.01$). FreeT₃-index and freeT₄-index were directly associated with HSS scores ($P < 0.01$). Stress scores were associated with HSS scores ($P < 0.01$) but not with biochemical severity of Graves' hyperthyroidism. Advancing age was associated with lower scores for stress exposure. Multivariate regression analysis showed that HSS score was independently related to the tendency to report negative feelings ($P < 0.01$) but not to other stress scores and also not to age.

Conclusion: Advancing age is associated with less exposure to stress, lower serum TBII and less severe clinical and biochemical Graves' hyperthyroidism. Because no direct relationship exists between stress exposure and TBII or freeT₃-index and freeT₄-index, we reject our hypothesis that less stress is causally related to biochemically less severe Graves' hyperthyroidism in old age. HSS score is primarily determined by negative feelings and not by age.

European Journal of Endocrinology 160 193–199

Introduction

Graves' hyperthyroidism is a multifactorial disease with many predisposing genetic and environmental factors. Seventy-nine percent of the susceptibility to develop Graves' hyperthyroidism can be attributed to genetic factors, leaving 21% for environmental factors (1). Exposure to stress, cigarette smoke, iodine excess, and several drugs have all been identified as relevant environmental factors in the pathogenesis of Graves' hyperthyroidism. For example, since the first description by Parry of an association between a stressful life event and the occurrence of hyperthyroidism in 1825, many studies have reported a greater number of stressful life

events in the year preceding the diagnosis of Graves' hyperthyroidism when compared with controls (2–6). The evidence that environmental stimuli may provoke Graves' hyperthyroidism in genetically susceptible subjects is rather good, but the quantitative relationship between the exposure to environmental stressors and the severity of the provoked thyrotoxicosis has scarcely been studied.

Age has consistently been observed as a significant modulating factor, advancing age being associated with less severe Graves' hyperthyroidism (7–9). The mechanism behind less severe Graves' hyperthyroidism in the older age groups is incompletely understood. Less severe Graves' hyperthyroidism is due to lower levels of

thyrotropin-binding inhibitory immunoglobulin (TBII) (10). Furthermore, it has been shown that advancing age is associated with a decreased experience of self-reported stress (pleasant and unpleasant life events) (11). In turn, psychological stress has a differential effect on immune response, suppressing cellular and potentiating humoral immunity (12). We hypothesize that advancing age is associated with less exposure to stress, resulting in lower production of TBII and thereby in less severe Graves' hyperthyroidism. To test this hypothesis, we performed a cross-sectional observational study in a large population of patients with newly diagnosed and untreated Graves' hyperthyroidism.

Subjects and methods

Study design

We included 263 consecutive untreated patients (69 males and 194 females, age 16–79 years at study entrance) with a first episode of Graves' hyperthyroidism in a cross-sectional, multicenter, observational study. Patients were included from nine participating centers in the Netherlands from July 2002 until September 2005. Inclusion criteria were biochemical hyperthyroidism ($\text{TSH} < 0.4 \text{ mU/l}$, $\text{fT}_4 > 23 \text{ pmol/l}$, and/or $\text{T}_3 > 2.7 \text{ nmol/l}$) and a diffuse homogeneous uptake on thyroid scintigraphy ($^{99\text{m}}\text{Tc}$ -pertechnetate). Exclusion criteria were relapse of Graves' hyperthyroidism, no written informed consent, no understanding of the Dutch language, and serious alcohol or i.v. drugs abuse. Clinical parameters at baseline like sex, age, goiter size (classification by means of WHO 1960 criteria) (13), hyperthyroid symptom scale score (HSS score), and the existence of pretibial myxedema and Graves' orbitopathy were recorded. Venous blood samples were taken for thyroid hormone measurements before treatment was started. The HSS score questionnaire (14) quantitatively measures clinical severity of hyperthyroidism through ten items on 0–4 point subscales, including sweating, heat intolerance, nervousness, hyperactivity, tremor, weakness, hyperdynamic precordium, diarrhea, appetite, and degree of incapacitation. Participants were asked to complete two stress and one mood questionnaires at time of diagnosis before treatment was started.

The study was approved by the local ethics committees of the Academic Medical Center of Amsterdam and the eight other participating centers. All patients gave written informed consent.

Stressful life event questionnaires

The Dutch questionnaire on recently experienced stressful life events (11) count the total number of major life events experienced in the past 12 months (checklist of 60 possible events). First, the respondent

scores the amount of pleasantness for each experienced life event on a scale from 0 to 4. Second, the respondent scores the amount of unpleasantness for each experienced life event in a similar manner on a scale from 0 to 4. Third, the total amount of pleasantness and the total amount of unpleasantness are separately calculated by summing up all scores for pleasantness and unpleasantness respectively (maximal score 240 for each). Fourth, the total number of pleasant events is calculated by counting the number of life events in which the score for pleasantness exceeds the score for unpleasantness. The total number of unpleasant events is calculated in a similar manner in which the score for unpleasantness exceeds the score for pleasantness (maximum 60).

The Dutch everyday problem checklist, a validated version of the daily hassles scale (15, 16), consists of 114 items concerning daily hassles experienced in the last 2 months. It also measures the intensity of each hassle on a scale from 0 to 3, yielding the number of hassles experienced and the total intensity of these hassles (maximum 342).

The Positive and Negative Affect Schedule (PANAS) (17) measures the current mood, in terms of positive and negative affect. It consists of 22 mood states (11 positive and 11 negative) and the respondent is asked to report whether he/she is affected by each of these states on a scale from 1 (not at all) to 5 (a lot). This yields the tendency to report positive and negative affect states both on a scale from 11 to 55.

Laboratory measurements

Non-fasting venous blood samples were taken for thyroid hormone measurements and were stored at 20 °C until assay. Serum triiodothyronine (T_3) and thyroxine (T_4) were measured with in-house RIA's (18). T_3 uptake was determined by a no extraction, solid phase ^{125}I RIA (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA, USA). Free T_4 -index and free T_3 -index were calculated by multiplying T_3 uptake with T_4 and T_3 respectively. Serum thyrotropin (TSH) was determined with a fluoroimmunoassay (Delfia hTSH, Perkin Elmer, Turku, Finland; detection limit 0.01 mU/l). Serum TBII was quantitatively determined by a second generation luminescence receptor assay (TRAK human LIA, BRAHMS, Berlin, Germany; detection limit 1.0 IU/l). Autoantibodies against thyroid peroxidase (TPO-Ab) were analyzed by anti-TPOn LIA (BRAHMS; cutoff levels < 30 and $> 3000 \text{ U/l}$). All measurements were performed at the laboratory of the Academic Medical Center of Amsterdam.

Statistical analysis

To analyze the influence of age on the severity of Graves' hyperthyroidism, patients were subdivided into four groups; ≤ 29 years ($N=53$), 30–39 years ($N=56$),

40–49 years ($N=78$), and ≥ 50 years ($N=76$). Regression analyses were performed between the four age groups and serum freeT₃-index, freeT₄-index, and HSS score. Trend analysis between different age groups, serum TBII, serum TPO-Ab, and stress scores was performed using the Jonckheere–Terpstra test. The association between different age groups, goiter size, and the prevalence of Graves' orbitopathy and pretibial myxedema was determined using χ^2 tests. Correlation coefficients (r) between serum thyroid hormones (freeT₃-index and freeT₄-index) and HSS scores were calculated by Spearman's rank-order correlation coefficient.

To evaluate the association between stress scores and the biochemical and clinical severity of Graves' hyperthyroidism, we subdivided all stress scores into quartiles. Linear regression analysis was performed to determine the association between stress scores and serum freeT₃-index, freeT₄-index, and HSS scores. For the association between stress scores and serum TBII we used the Jonckheere–Terpstra test. χ^2 test analysis was used for correlation analysis between stress scores and goiter size, the prevalence of pretibial myxedema and Graves' orbitopathy.

We used multivariate regression analysis to determine the effect of age and stress scores on the clinical severity of Graves' hyperthyroidism (HSS score).

$P<0.05$ indicated statistical significance. All statistical analyses were carried out using the SPSS statistical package for Windows, version 15.0. (SPSS Inc., Chicago, IL, USA).

Results

Characteristics of the study population are shown in Table 1. Male and female patients did not differ in age, or in biochemical and clinical severity of hyperthyroidism (data not shown).

Table 1 Baseline characteristics.

	N=263
Sex (male/female)	69/194
Age (years)	43 (32–51)
FreeT ₃ -index	7.0 (3.7)
FreeT ₄ -index	338 (124)
TBII (IU/l)	9.3 (5.0–18.8)
TPO-Ab (kU/l)	430 (40–2738)
Goiter size (%)	
0	64%
I	19%
II	14%
III	4%
Graves' orbitopathy (%)	19%
Pretibial myxedema (%)	5%
HSS score	15 (7)

Values for HSS score, serum freeT₃-index, and freeT₄-index are given as mean $\pm$ s.d. while values for age, serum TBII, and TPO-Ab are given as median $\pm$ interquartile range (range between the 25th and the 75th percentiles).

Table 2 shows the relationship between age and biochemical and clinical severity of Graves' hyperthyroidism at the time of diagnosis. Advancing age was associated with both biochemically less severe Graves' hyperthyroidism (freeT₃-index and freeT₄-index; $P<0.01$) and clinical less severe Graves' hyperthyroidism as measured by HSS scores (P for trend = 0.04). Trend analysis showed that serum TBII and to a lesser degree TPO-Ab decreased with advancing age ($P=0.05$ and $P=0.07$ respectively).

The HSS score was significantly associated with serum freeT₃-index ($r=0.28$, $P<0.01$) and to a lesser extent with freeT₄-index ($r=0.17$, $P<0.01$; Fig. 1). Goiter size decreased with advancing age (P for trend <0.01) (Table 2 and Fig. 2), while no association was found between age and the prevalence of orbitopathy and pretibial myxedema.

Table 3 shows the influence of stress scores on the biochemical and clinical severity of Graves' hyperthyroidism. Neither serum freeT₄-index, freeT₃-index nor TBII were associated with PANAS scores, frequencies and total scores for daily hassles and recently experienced stressful life events questionnaires except for the total amount of pleasantness that was directly associated with freeT₃-index (P for trend <0.01). By contrast, higher HSS scores were related to more negative mood and less positive mood on the PANAS affect scores ($P<0.01$). Higher HSS scores were also associated with higher frequencies and total scores for daily hassles except for the intensity per hassle and the recently experienced stressful life event questionnaires (Table 3).

None of the other clinical characteristics were associated with stress scores, with the exception of goiter size being positively correlated to both pleasantness and unpleasantness scores for recently experienced life events ($P<0.01$ and $P=0.01$ respectively) (data not shown).

Table 4 shows the associations between age and various stress scores. Advancing age was associated with lower PANAS negative affect scores (P for trend = 0.02) but not with PANAS positive affect scores ($P=0.75$). With regard to daily hassles, the frequencies and total scores for daily hassles decreased with advancing age (P for trend <0.01 and 0.01 respectively) while the intensity per hassle did not differ ($P=0.48$). Furthermore, advancing age was associated with lower frequencies and scores for the recently experienced life events questionnaire ($P<0.01$; Table 4).

To study the relationship between clinical severity (HSS score), age, and stress we performed multivariate regression analysis. The HSS score was used as dependent variable for all moods and stress scores (PANAS, The Dutch everyday problem checklist, and The Dutch questionnaire on recently experienced stressful life events) together with age (Table 5). This analysis showed that the clinical severity of Graves' hyperthyroidism was not related to age nor to the

Table 2 Age as determinant for biochemical and clinical severity of Graves' hyperthyroidism in 263 newly diagnosed patients.

	≤29 years (N=53)	30–39 years (N=56)	40–49 years (N=78)	≥50 years (N=76)	P value (trend)
Free T ₃ -index	8.3 (5.8)	7.2 (3.0)	6.5 (2.6)	6.5 (2.8)	<0.01
Free T ₄ -index	373 (156)	366 (107)	320 (116)	308 (107)	<0.01
TBII (IU/1)	11.7 (5.0–26.9)	9.0 (5.0–14.3)	10.2 (4.5–18.7)	7.7 (5.0–14.6)	0.05
TPO-Ab (kU/1)	655 (65–2922)	920 (80–>3000)	210 (40–2170)	265 (30–1582)	0.07
Goiter size (%)					<0.01
Grade 0	42%	56%	65%	83%	
Grade I	30%	22%	20%	7%	
Grade II	19%	14%	13%	10%	
Grade III	9%	7%	1%	0%	
Graves' orbitopathy (%)	13%	20%	20%	20%	0.38
Pretibial myxedema (%)	6%	4%	4%	5%	0.98
HSS score	16 (7)	16 (6)	15 (7)	14 (7)	0.04

Values for freeT₃-index, freeT₄-index, and HSS score are given as mean + s.d. (between parentheses). Values for TBII and TPO-Ab are given as median + interquartile range (range between the 25th and 75th percentiles).

amount of stress exposure but was independently related to the tendency to report negative feelings ($P < 0.01$).

Discussion

The first main finding of our study is that advancing age is associated with less severe Graves' hyperthyroidism (Table 2). FreeT₃-index and freeT₄-index are lower in the older age groups, as reported previously in several studies (7, 9, 19). Serum TBII also decreased with

advancing age, again in agreement with the literature (7). Because TBII is directly related to freeT₃-index and freeT₄-index (10), the fall in serum TBII provides a satisfactory explanation of why the biochemical severity of Graves' hyperthyroidism decreases in older age. We also observed a decrease in the clinical severity of Graves' hyperthyroidism with age as evident from the fall in the HSS score. There was furthermore a shift toward smaller goiter size in elderly patients, a finding again explainable from the direct relationship between TBII and goiter size (10). Other studies also reported a lower frequency of symptoms and signs in elderly Graves' hyperthyroidism patients (notably less heat tolerance, less perspiration, less irritability, and less often increased appetite, tachycardia and goiter), although weight loss and atrial fibrillation are more common in old age (20–22). Clinical severity (HSS score) was directly related to the biochemical severity (freeT₃-index and freeT₄-index) of Graves' hyperthyroidism, but the correlation is weak (Fig. 1). Trzepacz *et al.*

Figure 1 Correlation between biochemical severity ((a) freeT₃-index and (b) freeT₄-index) and clinical severity (HSS score) of thyrotoxicosis in untreated Graves' hyperthyroidism.

Figure 2 Goiter size as a function of age (goiter size grade 0, thyroid not or distinctly palpable but usually not visible with head in a normal or raised position; grade I, thyroid easily palpable and visible with the head in either a normal or raised position; grade II, thyroid easily visible with the head in a normal position; grade III, goiter visible at a distance).

Table 3 PANAS, daily hassles and recently experienced life events scores as determinants for biochemical (thyrotropin-binding inhibitory immunoglobulin, freeT₄-index, and freeT₃-index) and clinical (hyperthyroid symptom scale score) severity of Graves' hyperthyroidism.

	FreeT ₄ - index	FreeT ₃ - index	TBII (IU/l)	HSS score
PANAS negative affect score				
≤16	376 (143)	7.9 (5.4)	9.8 (5.6–24.2)	11 (6)
17–22	302 (97)	6.5 (2.6)	8.8 (4.4–18.4)	15 (7)
23–28	332 (128)	6.6 (3.2)	10.1 (4.8–18.6)	15 (7)
≥29	320 (98)	6.7 (3.3)	9.1 (4.0–16.0)	18 (7)
<i>P</i> value for trend	NS	NS	NS	<0.01
PANAS positive affect score				
≤32	325 (92)	7.1 (3.3)	7.9 (5.2–14.4)	16 (8)
33–38	350 (134)	7.2 (3.1)	10.2 (5.4–21.8)	15 (7)
39–41	305 (115)	6.4 (4.2)	6.9 (3.8–16.2)	14 (6)
≥42	333 (118)	6.7 (3.9)	11.7 (7.0–25.2)	13 (7)
<i>P</i> value for trend	NS	NS	NS	<0.01
Total number of daily hassles				
≤11	363 (132)	7.3 (4.3)	10.0 (5.7–18.3)	13 (7)
12–21	313 (101)	6.2 (2.7)	7.9 (4.2–17.0)	14 (7)
22–33	323 (126)	7.3 (3.8)	10.3 (5.6–21.5)	15 (8)
≥34	321 (106)	6.7 (3.6)	8.6 (4.2–16.2)	17 (6)
<i>P</i> value for trend	NS	NS	NS	<0.01
Intensity per hassle				
≤1.20	359 (145)	7.5 (4.3)	8.5 (5.0–18.6)	14 (6)
1.21–1.59	318 (119)	6.8 (4.0)	9.4 (5.0–22.5)	15 (8)
1.60–1.99	336 (103)	7.2 (3.4)	10.2 (4.8–17.6)	16 (7)
≥2.00	308 (95)	6.1 (2.6)	8.2 (4.7–13.9)	15 (8)
<i>P</i> value for trend	NS	NS	NS	NS
Daily hassles total score				
≤14	367 (138)	7.4 (4.4)	9.6 (5.4–19.0)	14 (7)
15–30	310 (101)	6.1 (2.4)	9.2 (3.6–15.6)	13 (7)
31–57	327 (114)	7.1 (3.7)	9.6 (6.7–19.7)	16 (8)
≥58	313 (107)	6.8 (3.8)	8.8 (4.2–19.4)	17 (6)
<i>P</i> value for trend	NS	NS	NS	<0.01
Number of life events				
≤4	330 (123)	6.2 (2.9)	8.0 (4.8–14.1)	13 (7)
5–8	335 (124)	7.0 (4.4)	10.0 (4.6–21.0)	14 (7)
9–14	314 (105)	7.1 (2.7)	9.4 (5.7–18.8)	15 (7)
≥15	335 (113)	7.2 (4.0)	9.7 (4.9–20.7)	17 (6)
<i>P</i> value for trend	NS	NS	NS	<0.01
Number of unpleasant life events				
≤2	328 (131)	6.7 (3.8)	8.0 (4.4–15.4)	13 (7)
3–5	344 (102)	7.0 (2.6)	9.3 (5.6–20.0)	14 (7)
6–8	311 (122)	7.0 (4.5)	10.2 (4.4–19.1)	15 (7)
≥9	324 (114)	6.9 (4.1)	9.2 (4.8–19.7)	17 (7)
<i>P</i> value for trend	NS	NS	NS	<0.01
Total score unpleasantness				
≤6	335 (119)	6.5 (2.8)	8.0 (5.0–12.2)	13 (7)
7–15	336 (116)	7.0 (3.5)	10.2 (4.5–20.0)	14 (7)
16–28	315 (122)	6.9 (4.2)	9.3 (5.4–20.3)	15 (7)
≥29	329 (113)	7.2 (4.0)	9.7 (4.8–20.7)	17 (7)
<i>P</i> value for trend	NS	NS	NS	<0.01
Number of pleasant life events				
≤1	328 (117)	6.6 (3.9)	8.0 (4.2–12.4)	14 (7)
2	310 (111)	6.2 (2.5)	9.9 (5.6–21.0)	13 (7)
3–5	325 (107)	7.4 (3.4)	10.2 (6.1–22.5)	16 (7)
≥6	352 (133)	7.4 (4.3)	10.5 (4.3–21.7)	16 (7)

Table 3 Continued

	FreeT ₄ - index	FreeT ₃ - index	TBII (IU/l)	HSS score
<i>P</i> value for trend	NS	NS	NS	0.03
Total score pleasantness				
≤3	337 (113)	6.2 (2.7)	8.0 (3.7–14.6)	12 (7)
4–9	320 (127)	6.2 (3.9)	8.8 (5.2–17.0)	14 (7)
10–17	312 (96)	6.8 (2.6)	9.9 (5.8–18.7)	16 (7)
≥18	354 (125)	7.9 (4.7)	11.5 (4.3–24.3)	17 (7)
<i>P</i> value for trend	NS	<0.01	NS	<0.01

All stress scores were subdivided into quartiles. Values for serum freeT₃-index, freeT₄-index, and HSS score are given as mean+s.d. (between parentheses). Values for serum TBII are given as median + interquartile range (between parentheses).

did not observe a relationship between the HSS score and serum thyroid hormone concentrations in newly diagnosed untreated Graves' hyperthyroidism patients (23), most likely due to the low sample size ($n=25$) in their study.

The second main finding of our study is that overall stress exposure is not related to the biochemical severity of Graves' hyperthyroidism, but, by contrast, is directly related to the clinical severity (HSS score) of Graves' hyperthyroidism (Table 3). Affect scores and stress from daily hassles or unpleasant recent life events were not related to freeT₃-index and freeT₄-index or to TBII; only higher amounts of pleasantness were significantly related to freeT₃-index. By contrast, all stress parameters were associated with the clinical severity of Graves' hyperthyroidism: higher negative affect or lower positive affect scores, and higher frequencies and scores for daily hassles and (un)pleasantness of recent life events were all associated with higher HSS scores. How can we explain the discrepancy that stress scores are only related to clinical severity and not to biochemical severity of Graves' hyperthyroidism? When a person contracts a disease, his complaints to some extent will be influenced by his current mood state. If the person scores high on negative affects or low on positive affects, he/she is more likely to have complaints or to rate a particular complaint as more severe. Indeed, our multivariate regression analysis demonstrated that the HSS score was independently related to the tendency to report negative feelings ($P<0.01$) and not to any of the other stress scores, nor to age (Table 5).

The third main finding of our study is that advancing age is associated with less exposure to stress (Table 4). The frequency and total amount of unpleasantness or pleasantness experienced from stressful life events in the year preceding the diagnosis of Graves' hyperthyroidism decreased in the older age groups, and the same was true for the frequency and total intensity of daily hassles of the last months. The current mood state of elderly patients was also better, because they were less influenced by negative affects. These interesting observations are in line with studies from the field of

Table 4 Age as determinant for the amount of stress in 263 newly diagnosed patients with Graves' hyperthyroidism.

	≤29 years (N=53)	30–39 years (N=56)	40–49 years (N=78)	≥50 years (N=76)	P value (trend)
Positive and negative affect schedule (PANAS)					
Negative affect score	26.5 (18.2–0.0)	25.5 (18.2–31.8)	22.0 (17.0–28.0)	21.0 (16.0–26.0)	0.02
Positive affect score	38.5 (33.2–41.0)	37.5 (32.2–41.0)	38.5 (33.0–43.0)	38.0 (33.0–40.0)	0.75
Everyday problem checklist					
Total number of daily hassles	26.0 (17.0–40.0)	27.0 (12.0–39.0)	18.0 (10.5–28.9)	19.4 (10.0–29.0)	<0.01
Intensity per hassle	1.6 (1.3–1.9)	1.6 (1.2–1.9)	1.6 (1.2–2.0)	1.8 (1.1–2.1)	0.48
Daily hassles total score	41.0 (22.0–69.0)	36.3 (16.0–63.0)	26.0 (13.0–48.5)	28.0 (9.0–48.0)	0.01
Recently experienced stressful life events					
Number of life events	13.0 (7.0–20.0)	9.0 (7.0–15.0)	6.0 (4.0–10.5)	7.0 (4.0–12.0)	<0.01
Number of unpleasant life events	7.0 (3.0–10.0)	6.0 (3.0–11.0)	4.0 (2.0–7.0)	4.0 (2.0–7.0)	<0.01
Total score unpleasantness	22.0 (11.0–44.0)	19.0 (10.0–38.0)	12.0 (6.0–19.0)	13.0 (4.0–23.5)	<0.01
Number of pleasant life events	5.0 (3.0–10.0)	3.0 (1.0–6.0)	2.0 (1.0–3.0)	2.0 (0.0–3.0)	<0.01
Total score pleasantness	21.0 (11.0–30.0)	13.0 (7.0–22.0)	8.0 (3.5–11.5)	6.0 (2.5–11.0)	<0.01

Values are given as median + interquartile range (between parentheses).

psychology on stress and aging. Older adults reported fewer undesirable daily events than do younger adults (24). When stressors do occur, older age is related to reductions in perceiving severity and affective distress (25, 26).

If stress is not involved in lower TBII and consequently in freeT₃-index and freeT₄-index levels observed in old age, the question arises which other mechanisms might be responsible. Most likely it is the decline in immunological response to antigens with age, a process called immunosenescence (27). Animal models on experimental autoimmune thyroiditis have shown that lymphocytes from old mice are less effective in transferring thyroiditis than lymphocytes from young donors (28, 29). A recent study indicates that in the elderly the B-cell repertoire available to respond to new antigenic challenge is decreased (30). The shift away from predominantly naïve B-cells with time obviously reflects the influence of cumulative exposure to foreign

pathogens over time. It means that advancing age is associated with a lack of clonotypic immune response to antigens.

Going back to our hypothesis we can conclude that advancing age is indeed associated with less exposure to stress, lower serum TBII and less severe clinical and biochemical Graves' hyperthyroidism. Because no direct relationship existed between stress exposure and TBII or freeT₃-index and freeT₄-index, we reject our hypothesis that less stress is causally related to biochemically less severe Graves' hyperthyroidism in old age. We find the observed tendency to higher freeT₃-index with higher amounts of total pleasantness insufficient evidence to support our hypothesis in view of all other findings. Because the extent to which patients express complaints are influenced by many factors including current mood state, we prefer freeT₃-index and freeT₄-index as objective measures for the severity of Graves' hyperthyroidism.

Declaration of interest

All authors declare that we do not have any financial or other potential conflict of interest; and also declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

We gratefully thank the participating physicians for their collaboration in this study: Dr A B Arntzenius (Spaarnse Ziekenhuis, Hoofddorp), Dr C B Brouwer (Onze Lieve Vrouwe Gasthuis, Amsterdam), Dr J Derksen (Medisch Spectrum Twente, Enschede), Dr M J M Diekman, and Dr M N Gerding (Ziekenhuis Deventer, Deventer), Dr V E A Gerdes (Slotervaartziekenhuis, Amsterdam), Dr W E de Graaff (Tergooiziekenhuizen, Blaricum), Dr R Heijligenberg (Ziekenhuis Gelderse Vallei, Ede), and Dr A F Muller (Diakonessenhuis, Utrecht). We would like to thank Ms E M Johannesma for all laboratory measurements.

Table 5 Multivariate regression analysis for the effect of stress scores and age on clinical severity (HSS score) of Graves' hyperthyroidism.

	Coefficient	P value
Constant	13.896 (4.036)	<0.01
Age	−0.041 (0.040)	0.307
Positive and negative affect schedule (PANAS)		
Negative affect score	0.233 (0.067)	<0.01
Positive affect score	−0.106 (0.065)	0.106
Everyday problem checklist		
Total number of daily hassles	0.110 (0.083)	0.187
Intensity per hassle	−0.508 (1.187)	0.669
Daily hassles total score	−0.030 (0.051)	0.548
Recently experienced stressful life events		
Number of life events	−0.396 (0.467)	0.398
Number of unpleasant life events	0.271 (0.583)	0.643
Total score unpleasantness	0.044 (0.121)	0.715
Number of pleasant life events	0.467 (0.535)	0.384
Total score pleasantness	0.026 (0.184)	0.890

Standard error (between parentheses).

References

- 1 Brix TH, Kyvik KO, Christensen K & Hegedus L. Evidence for a major role of heredity in Graves' disease: a population-based study of two Danish twin cohorts. *Journal of Clinical Endocrinology and Metabolism* 2001 **86** 930–934.
- 2 Graves RJ. New observed affection of the thyroid gland in females. *London Medical and Surgical Journal* 1835 **7** 516–517.
- 3 Parry C. *Collections From the Unpublished Writings of the Late CH Parry*. London: Underwoods, 1825.
- 4 Paunkovic N, Paunkovic J, Pavlovic O & Paunovic Z. The significant increase in incidence of Graves' disease in eastern Serbia during the civil war in the former Yugoslavia (1992 to 1995). *Thyroid* 1998 **8** 37–41.
- 5 von Basedow KA. Exophthalmus durch Hypertrophie des Zellgewebes in der Augenhöhle. *Wochenschrift für Die Gesamte Heilkunde* 1840 **6** 197–204.
- 6 Winsa B, Adami HO, Bergstrom R, Gamstedt A, Dahlberg PA, Adamson U, Jansson R & Karlsson A. Stressful life events and Graves' disease. *Lancet* 1991 **338** 1475–1479.
- 7 Aizawa T, Ishihara M, Hashizume K, Takasu N & Yamada T. Age-related changes of thyroid function and immunologic abnormalities in patients with hyperthyroidism due to Graves' disease. *Journal of the American Geriatrics Society* 1989 **37** 944–948.
- 8 Kawabe T, Komiya I, Endo T, Koizumi Y & Yamada T. Hyperthyroidism in the elderly. *Journal of the American Geriatrics Society* 1979 **27** 152–155.
- 9 Manji N, Carr-Smith JD, Boelaert K, Allahabadia A, Armitage M, Chatterjee VK, Lazarus JH, Pearce SHS, Vaidya B, Gough SC & Franklyn JA. Influences of age, gender, smoking, and family history on autoimmune thyroid disease phenotype. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 4873–4880.
- 10 Vos XG, Smit N, Enderit E, Tijssen JG & Wiersinga WM. Frequency and characteristics of TBII-seronegative patients in a population with untreated Graves' hyperthyroidism: a prospective study. *Clinical Endocrinology* 2008 **69** 311–317.
- 11 van de Willige G, Schreurs P, Telligen B & Zwart F. Het meten van 'life events': Vragenlijst Recent Meegemaakte Gebeurtenissen. *Nederlands Tijdschrift Voor de Psychologie en Haar Grensgebieden* 1985 **40** 1–19.
- 12 Tsatsoulis A. The role of stress in the clinical expression of thyroid autoimmunity. *Annals of the New York Academy of Sciences* 2006 **1088** 382–395.
- 13 Perez C, Scrimshaw NS & Munoz JA. Technique of endemic goitre surveys. *Monograph Series. World Health Organization* 1960 **44** 369–383.
- 14 Klein I, Trzepacz PT, Roberts M & Levey GS. Symptom rating scale for assessing hyperthyroidism. *Archives of Internal Medicine* 1988 **148** 387–390.
- 15 Kanner AD, Coyne JC, Schaefer C & Lazarus RS. Comparison of two modes of stress measurement: daily hassles and uplifts versus major life events. *Journal of Behavioral Medicine* 1981 **4** 1–39.
- 16 Vingerhoets AJJM, Jeninga AJ & Menges LJ. Het meten van chronische en alledaagse stressoren: II. Eerste onderzoekservaringen met de Alledaagse Problemenlijst (APL). The measurement of daily hassles and chronic stressors: the development of the Everyday Problem Checklist (EPCL), Dutch: APL. *Gedrag & Gezondheid* 1989 **17** 10–17.
- 17 Watson D, Clark LA & Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *Journal of Personality and Social Psychology* 1988 **54** 1063–1070.
- 18 Wiersinga WM & Chopra IJ. Radioimmunoassay of thyroxine (T₄), 3,5,3'-triiodothyronine (T₃), 3,3',5'-triiodothyronine (reverse T₃, rT₃), and 3,3'-diiodothyronine (T₂). *Methods in Enzymology* 1982 **84** 272–303.
- 19 Allahabadia A, Daykin J, Holder RL, Sheppard MC, Gough SCL & Franklyn JA. Age and gender predict the outcome of treatment for Graves' hyperthyroidism. *Journal of Clinical Endocrinology and Metabolism* 2000 **85** 1038–1042.
- 20 Mariotti S, Franceschi C, Cossarizza A & Pinchera A. The aging thyroid. *Endocrine Reviews* 1995 **16** 686–715.
- 21 Nordyke RA, Gilbert FI Jr & Harada AS. Graves' disease. Influence of age on clinical findings. *Archives of Internal Medicine* 1988 **148** 626–631.
- 22 Trivalle C, Doucet J, Chassagne P, Landrin I, Kadri N, Menard JF & Bercoff E. Differences in the signs and symptoms of hyperthyroidism in older and younger patients. *Journal of the American Geriatrics Society* 1996 **44** 50–53.
- 23 Trzepacz PT, Klein I, Roberts M, Greenhouse J & Levey GS. Graves' disease: an analysis of thyroid hormone levels and hyperthyroid signs and symptoms. *American Journal of Medicine* 1989 **87** 558–561.
- 24 Zautra AJ, Finch JF, Reich JW & Guarnaccia CA. Predicting the everyday life events of older adults. *Journal of Personality* 1991 **59** 507–538.
- 25 Phifer JF. Psychological distress and somatic symptoms after natural disaster: differential vulnerability among older adults. *Psychology and Aging* 1990 **5** 412–420.
- 26 Uchino BN, Berg CA, Smith TW, Pearce G & Skinner M. Age-related differences in ambulatory blood pressure during daily stress: evidence for greater blood pressure reactivity with age. *Psychology and Aging* 2006 **21** 231–239.
- 27 Prelog M. Aging of the immune system: a risk factor for autoimmunity? *Autoimmunity Reviews* 2006 **5** 136–139.
- 28 Okayasu I, Hatakeyama S & Kong YC. Long-term observation and effect of age on induction of experimental autoimmune thyroiditis in susceptible and resistant mice. *Clinical Immunology and Immunopathology* 1989 **53** 254–267.
- 29 Romball CG & Weigle WO. The effect of aging on the induction of experimental autoimmune thyroiditis. *Journal of Immunology* 1987 **139** 1490–1495.
- 30 Listi FLOR, Candore GIUS, Modica MA, Russo MARI, Lorenzo GD, Esposito-Pellitteri MARI, Colonna-Romano GIUS, Aquino ALES, Bulati MATT, Lio DOME, Franceschi CLAU & Caruso CALO. A study of serum immunoglobulin levels in elderly persons that provides new insights into B cell immunosenescence. *Annals of the New York Academy of Sciences* 2006 **1089** 487–495.

Received 9 October 2008

Accepted 27 October 2008