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Involvement of stress in the pathogenesis of autoimmune thyroid disease: A prospective study

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Daily hassles;
Mood

Summary

Background: An association between stress and autoimmune thyroid disease (AITD) (especially Graves' hyperthyroidism) has been reported, but all studies so far on this topic have been retrospective.

Objective: To evaluate prospectively the relationship between stress and (i) de novo occurrence of thyroid antibodies and (ii) development of overt autoimmune hyper-/hypothyroidism.

Study design: Two nested case—control studies in a prospective cohort of 790 euthyroid women who were 1st or 2nd degree relatives of AITD patients. Follow-up was five year, with annual assessments including questionnaires on stressful life events, daily hassles, and mood. In study A, cases were subjects who developed TPO-Ab but remained euthyroid during follow-up (called event). In study B, cases were subjects who developed overt hypothyroidism (TSH $>$ 5.7 mU/l and FT4 $<$ 9.3 pmol/l) or overt hyperthyroidism (TSH $<$ 0.4 mU/l and FT4 $>$ 20.1 pmol/l) during follow-up (called event). For each case, two controls were selected, matched for age and duration of follow-up; controls in study A remained TPO-Ab negative, and in study B remained without overt hyper-/hypothyroidism.

Outcomes: Contrast in questionnaire responses between cases and controls at baseline, at one year prior to the event and at time of event.

Results: Exposure to stress was not different between subjects who developed or did not develop TPO-Ab (study A). No differences were observed in stress questionnaires between hyper-/hypothyroid cases and controls at any time point, but hypothyroid cases had less negative feelings than controls at the time of diagnosis (study B).

Conclusion: The data suggest that stress is not involved in the pathogenesis of AITD.

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1. Introduction

Autoimmune thyroid disease (AITD) results from a complex interplay among genetic and environmental factors. Evidence for a genetic base of this disease is strong as siblings and other family members of AITD patients are at increased risk for AITD (Chopra et al., 1977; Jacobson and Tomer, 2007). The sibling recurrence risk ratio (λ_s) is 11.6 for Graves' and 28.0 for Hashimoto's disease (Villanueva et al., 2003). In women with at least one first degree relative with AITD, the incidence of Graves' hyperthyroidism is 4.1—4.3 times higher and of Hashimoto's hypothyroidism 1.9—2.7 times higher than the general female population (Strieder, 2008). Twin studies suggest that genetic factors play a major role, accounting for about 70% of the risk to get AITD (Brix et al., 2000, 2001; Tomer and Huber, 2009). The remaining 30% must be due to environmental factors, which alter the immune reaction depending on the duration and intensity of exposure (Prummel et al., 2004). Among those factors is stress.

Stress affects the immune system both directly and indirectly through the activation of neural and endocrine systems (Chrousos, 1998; Elenkov, 2004). The phenotypic expression of AITD is to a large extent dependent on the balance of Th1 versus Th2 immune response (Weetman, 2004). During periods of stress an increase of the secretion of glucocorticoids and catecholamines is observed as a result of the activation of the sympathoadrenal system and the hypothalamic—pituitary—adrenal axis, respectively. Both glucocorticoids and catecholamines cause a selective suppression of Th1 response and a shift toward Th2 mediated humoral immunity (Chrousos and Elenkov, 2006). This mechanism may promote the development of Graves' disease, which is a Th2 predominant disease (Tsatsoulis, 2006). On the other hand, a hypoactive hypothalamic—pituitary—adrenal axis may leads to a predominantly Th1-mediated immune activity (Wilder, 1995), which may promote thyroid cell destruction and Hashimoto's thyroiditis through apoptotic pathways on thyroid follicular cells.

The natural history of AITD probably starts with a particular genetic background and then several stages can be distinguished. The first stage in the natural history of thyroid autoimmunity that can be detected is the occurrence of TPO-Ab. Thereafter, changes in serum TSH at values outside the normal range follow (subclinical hypo- or hyperthyroidism) and the disease ends in many instances with overt hypo- or hyperthyroidism. In order to get more insight in the influence of stress on the natural course of AITD we did two nested case—control studies within the Amsterdam AITD cohort. The aim of the first study was to evaluate the relationship between stress and the de novo occurrence of TPO-Ab and the aim of the second study was to evaluate the relationship between stress and the development of overt hypothyroidism or overt hyperthyroidism.

2. Subjects and methods

2.1. Participants

The present study was carried out among the 803 subjects from the Amsterdam AITD Cohort. The original cohort has previously been described in detail (Strieder et al., 2003). In short, the cohort consisted of women between 18 and 65 years of age in self proclaimed good health without a history of thyroid disease, who had at least one 1st or 2nd degree relative with documented autoimmune hyper- or hypothyroidism. Subjects were followed for five years, or shorter when overt hyper- or hypothyroidism had occurred (defined as TSH $<$ 0.4 mU/l in combination with FT4 $>$ 20.1 pmol/l, or TSH $>$ 5.7 mU/l in combination with FT4 $<$ 9.3 pmol/l, respectively). At each annual visit blood samples were collected to measure TSH, FT4, T3, TPO-Ab, Tg-Ab and TBII, and subjects were asked to fill a number of questionnaires on stressful life events, daily hassles, and mood.

The Dutch questionnaire on Recent Experienced Stressful Life Events (van de Willige et al., 1985; Brosschot et al., 1994) counts the total number of major life events experienced in the last 12 months (checklist of 60 possible events). The respondent scores separately the amount of pleasantness and unpleasantness with each experienced life event, rated on a scale of zero (meaning no (un)pleasantness at all) to four (a huge amount of (un)pleasantness). From this scale, the total amount of pleasantness and unpleasantness is calculated (maximum score 240 for each). When the amount of pleasantness exceeds the amount of unpleasantness the event is categorized as being pleasant and vice versa, yielding the total number of (un)pleasant events (maximum 60). The Dutch Everyday Problem Checklist, a validated version of the daily hassles scale (Kanner et al., 1981; Vingerhoets et al., 1989, 1996), consists of 114 items concerning daily hassles in the last two months. It also measures the intensity of each hassle on a scale from zero to three, yielding the number of hassles experienced and the total intensity of these hassles (maximum 342). The Positive and Negative Affect Schedule (PANAS, Watson et al., 1988) measures the current mood, in terms of positive and negative affect. It consists of 22 mood states (11 positive, 11 negative) and the respondent is asked to report whether 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.

Results of thyroid function tests at study entrance revealed overt hypothyroidism in 10 subjects and overt hyperthyroidism in 3 subjects, leaving 790 subjects to be included in the present study. Within this cohort we performed two nested case—control studies. In both studies, for each case two controls were selected. Controls were matched by age and duration of follow-up. Matching by age was done in view of the increase in prevalence of thyroid antibodies with advancing age (Hollowell et al., 2002). A subject could only be sampled once as control. From all possible subjects who could serve as candidate controls, we selected those that were closest to the corresponding case, first for age and then for the follow-up period.

Study A. In order to evaluate the relationship between stress and the de novo occurrence of TPO-Ab, we selected participants from the original cohort as follows (Fig. 1): from the 790 euthyroid at study entrance subjects, we excluded those who had thyroid antibodies (i.e. serum concentrations of either TPO-Ab of 100 kU/l or greater, Tg-Ab of 100 kU/l or greater, or TBII of 12 U/l or greater). From the remaining 549 euthyroid subjects without thyroid antibodies we subsequently excluded those who had subclinical hyper- or

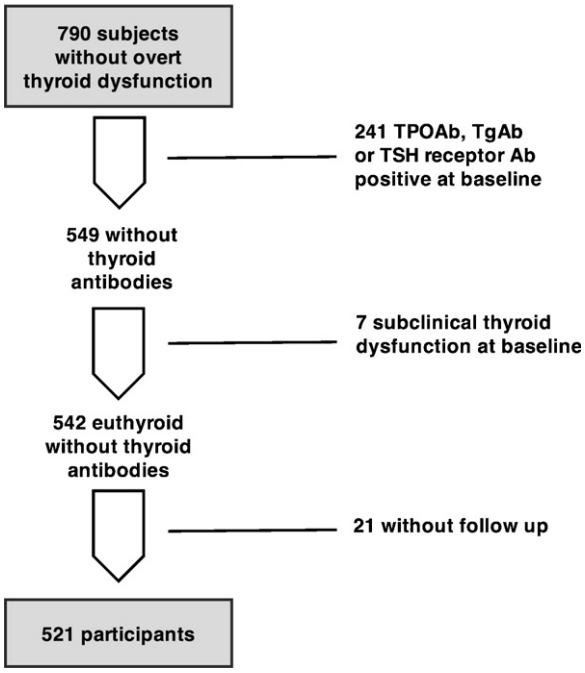

Figure 1 Flowchart of the recruitment of subjects for study A.

hypothyroidism (abnormal TSH in the presence of normal FT4 and T3) and those who had no follow-up. Five hundred twenty-one euthyroid participants without any serological sign of AITD at baseline were thus enrolled. In this study, a subject was recruited as a case when she remained euthyroid but had developed TPO-Ab. The end-point for a case was the time at which she had become positive for the first time for TPO-Ab without developing abnormal TSH (called event). Controls should have remained euthyroid and seronegative for TPO-Ab up to the time at which the case they were matched to, had received her end-point.

Study B. In order to evaluate the relationship between stress and the development of overt hypothyroidism or overt hyperthyroidism, we designed a nested case—control study among the 790 subjects euthyroid at study entrance. A subject was recruited as a case when she had developed over hypothyroidism or overt hyperthyroidism during follow-up (called event). The end-point for a case was the time at which she had developed overt hypo- or hyperthyroidism. Controls should have normal TSH up to the time at which the case they were matched to, had received her end-point.

Stress measurements at baseline, in the year prior to and at the time of the seroconversion to TPO-Ab for study A and the development of overt hypo- or hyper-thyroidism for study B were compared between cases and controls.

2.2. Laboratory measurements

Serum TSH and FT4 were measured using time-resolved fluoroimmunoassay (Delphia, Turku, Finland). Reference values are for TSH 0.4—5.7 mU/l and for FT4 9.3—20.1 pmol/l. Thyroid peroxidase (TPO) antibodies and thyroglobulin (Tg) antibodies were measured by chemiluminescence immunoassays (LUMI-test anti-TPO and LUMI-test anti-Tg, respectively, Brahms, Berlin, Germany). Improved versions of both assays became available during follow-up: detection limits of these new assays were for TPO-Ab 30 kU/l and for Tg-Ab 20 kU/l. TPO-Ab concentrations obtained with the old assay were multiplied by a factor 0.72 to obtain comparative values in the new assay. TPO-Ab and Tg-Ab concentrations were considered to be positive at values $\geq$100 kU/l. TSH receptor antibodies were determined as TSH binding inhibitory immunoglobulins (TBII) using the TRAK assay (Brahms, Berlin, Germany); detection limits in the 1st and 2nd generation TRAK assays were 5 and 1 IU/l, respectively, and values above 12 and 1.5 U/l, respectively were considered as positive.

3. Statistical analysis

Values are given as mean $\pm$ SD for age and follow-up period but as median and interquartile range for TSH and TPO-Ab. Differences between cases and controls were evaluated by Mann—Whitney *U*-test for stress parameters. A *p*-value of <0.05 was considered to indicate significant differences between groups.

4. Results

4.1. Study A. Stress and development of TPO-Ab

During the 5-year follow-up period, 81 of the 521 subjects (15.5%) had developed TPO-Ab while their TSH remained normal (Table 1). The mean age of the converters to TPO-Ab was 36 $\pm$ 12 years and the mean follow-up was 2.8 $\pm$ 1.3 years. Controls mean age and mean follow-up did not differ from cases. At the time of seroconversion the TPO-Ab concentration had a median value of 140 kU/l (interquartile range 110—160 kU/l). Statistical analysis at baseline, at time one year prior the occurrence of event and at time of event revealed no significant differences or trends in reported stress between cases and controls with respect to any of the questionnaires (Table 2). The results for developing either TPO-Ab and/or Tg-Ab were essentially the same (see the table in the supplementary data on the journal website).

4.2. Study B. Stress and development of overt hypo- or hyperthyroidism

During the 5-year follow-up period 13 cases of overt autoimmune hyperthyroidism and 38 cases of overt autoimmune hypothyroidism occurred after a mean follow-up of 2.7 $\pm$ 1.5 and 3.2 $\pm$ 1.3, respectively (Table 1). The cause of overt hyperthyroidism was Graves disease in 11 subjects (1 had Graves ophthalmopathy), postpartum thyroiditis in 1 subject, and silent thyroiditis in 1 subject. All subjects with overt hyperthyroidism had positive TPO-Ab and/or Tg-Ab at the time of diagnosis; TSH receptor antibodies were absent in the two patients diagnosed with postpartum thyroiditis and silent thyroiditis, but present in 6 out of 7 patients diagnosed with Graves' hyperthyroidism (TBII was not measured in the time of diagnosis in 4 Graves' patients). TSH receptor antibodies were undetectable in all controls at each time point, and in all cases at baseline and at the year before the event. Cases and controls did not differ with regard to mean age and mean follow-up.

Table 1 Characteristics of subjects who developed TPO-Ab (study A) or overt autoimmune thyroid disease (study B) and their corresponding controls at time of study entrance and at consecutive time points of follow-up, in a nested case–control study of women with 1st or 2nd degree relatives with proven AITD derived from the prospective Amsterdam AITD cohort.

	N	Age, years (SD)	Follow-up, years (SD)	TSH (mU/l)		FT4 (pmol/l)		TPO-Ab (kU/l)		Tg-Ab (kU/l)	
				Baseline	At time of event	Baseline	At time of event	Baseline	At time of event	Baseline	At time of event
Study A											
Cases	81	36 (12)	2.8 (1.3)	1.5 (1.2–2.2)	1.5 (1.1–2.2)	13.1 (11.6–14.5)	13.1 (11.9–14.5)	<25 (<25–<25)	140 (110–160)	15 (<5–25)	17 (6–43)
Controls	162	36 (12)	2.8 (1.3)	1.5 (1.1–2.1)	1.4 (1.0–2.0)	13.1 (11.9–14.5)	13.1 (11.9–14.9)	<25 (<25–<25)	<25 (<25–50)	5 (<5–15)	6 (6–15)
p-Values[a]				0.95	0.17	0.79	0.99	0.89	<0.001	0.005	<0.001
Study B1 (hyperthyroidism)											
Cases	13	41 (14)	2.7 (1.5)	1.6 (1.1–2.2)	0.02 (0.01–0.13)	13.3 (12.0–14.1)	41.5 (33.5–56.0)	560 (<25–1940)	1310 (50–3000)	38(23–108)	175 (44–185)
Controls	26	41 (14)	2.7 (1.5)	1.5 (1.2–2.2)	1.9 (1.2–2.6)	14.1 (12.0–15.7)	13.0 (12.2–14.1)	<25 (<25–80)	40 (<25–50)	17 (<5–41)	26 (<5–115)
p-Values[a]				0.85		0.34	<0.001	<0.001	0.01	0.03	<0.001
Study B2 (hypothyroidism)											
Cases	38	38 (12)	3.2 (1.3)	4.2 (2.7–5.7)	14.8 (6.3–16.9)	10.7 (9.7–11.6)	7.1 (4.8–8.4)	1123 (304–2239)	3000 (1620–6670)	75 (40–238)	140 (100–500)
Controls	76	38 (12)	3.2 (1.2)	1.6 (1.1–2.5)	1.4 (1.0–2.1)	12.8 (11.3–14.5)	13.0 (11.8–15.1)	25 (<25–50)	25 (<25–170)	8 (<5–29)	11 (6–69)
p-Values[a]				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data are given as mean ± SD or median with interquartile range. Time of event: first occurrence of TPO-Ab (study A), or diagnosis of hyper- or hypothyroidism (study B). TSH: thyroid stimulating hormone, FT4: free T4, TPO: thyroid peroxidase, Tg: thyroglobulin.

[a] Cases versus controls.

Table 2 Comparison of scores on three questionnaires (recent experienced life events, daily hassles, PANAS) between patients who developed TPO-Ab and remained euthyroid (normal TSH) and their corresponding controls matched for age and follow-up, in a nested case–control study of 243 women with 1st or 2nd degree relatives with proven AITD derived from the prospective Amsterdam AITD cohort.

	Baseline		One year before event		At event		*p*-Value		
	Cases	Controls	Cases	Controls	Cases	Controls	Baseline	One year before event	At event
Positive and negative affect schedule scale (tendency to report)									
Positive feelings	39.0 (35.0–42.0)	38.0 (34.0–42.0)	38.0 (35.0–41.0)	38.0 (34.0–41.0)	37.0 (34.0–41.0)	37.0 (34.0–41.0)	0.598	0.244	0.499
Negative feelings	22.0 (17.0–28.0)	21.0 (16.0–28.0)	21.0 (16.0–26.0)	22.00 (15.0–27.0)	20.0 (16.0–27.0)	20.00 (15.0–27.0)	0.483	0.622	0.532
Recent life events									
Total number	10.0 (7.0–15.0)	11.0 (7.0–15.0)	9.0 (5.0–15.0)	9.0 (6.0–14.0)	8.0 (5.0–13.0)	9.0 (5.0–13.0)	0.381	0.607	0.361
Number of unpleasant life events	5.0 (3.0–7.0)	5.0 (3.0–7.0)	4.0 (2.0–6.1)	3.0 (2.0–6.0)	3.0 (1.0–6.0)	3.0 (2.0–6.0)	0.577	0.488	0.640
Number of pleasant life events	5.0 (3.0–6.0)	4.0 (3.0–7.0)	4.0 (2.0–7.0)	4.0 (2.0–6.0)	3.0 (1.0–5.0)	4.0 (2.0–6.0)	0.756	0.730	0.357
Amount of total unpleasantness	15.0 (7.0–23.0)	17.0 (9.0–25.0)	12.0 (6.0–23.39)	12.0 (7.0–21.0)	12.0 (5.0–20.1)	13.0 (6.0–21.0)	0.387	0.915	0.634
Amount of total pleasantness	16.0 (9.0–23.0)	16.0 (9.15–24.0)	15.0 (7.0–24.0)	13.0 (8.0–22.0)	13.0 (6.0–20.0)	13.0 (6.0–21.0)	0.596	0.672	0.573
Daily hassles									
Total number of daily hassles	22.0 (17.0–35.0)	23.0 (14.0–35.0)	25.5 (15.5–37.5)	22.0 (15.0–35.0)	25.5 (14.0–36.0)	23.0 (14.0–36.0)	0.595	0.328	0.621
Intensity per hassle	1.36 (1.12–1.78)	1.32 (1.0–1.56)	1.40 (1.11–1.78)	1.33 (1.0–1.61)	1.35 (1.03–1.62)	1.33 (1.07–1.65)	0.069	0.089	0.679
Total intensity of all hassles	30.0 (20.0–52.0)	29.0 (15.1–50.0)	33.0 (20.0–55.5)	30.0 (14.0–51.0)	33.0 (18.0–54.0)	29.0 (16.0–51.0)	0.222	0.097	0.419

Data are given as median with interquartile range between parentheses. *p*-Value of cases versus controls. Time of event: visit when diagnosis of overt hyper- or hypothyroidism was made or confirmed. PANAS: positive and negative affect schedule.

Table 3 Comparison of scores on three stress questionnaires (recent experienced life events, daily hassles, PANAS) between patients who developed overt auto-immune thyroid disease (AITD) and their corresponding controls matched for age and follow-up.

	Baseline		One year before event		At event		*p*-Value		
	Cases	Controls	Cases	Controls	Cases	Controls	Baseline	One year before event	At event
Hyperthyroism study B1									
Positive and negative affect schedule scale (tendency to report)									
Positive feelings	39.0 (38.0—41.0)	38.0 (35.0—39.0)	36.0 (34.0—40.0)	37.0 (34.0—40.0)	36.0 (30.5—41.5)	37.0 (34.0—39.0)	0.05	0.70	0.93
Negative feelings	22.0 (19.0—26.0)	19.0 (15.0—29.0)	20.0 (15.0—22.0)	19.0 (15.0—26.0)	19.0 (15.0—25.0)	18.0 (13.0—22.0)	0.48	0.64	0.72
Recent life events									
Total number	7.0 (7.0—9.0)	9.5 (6.0—14.0)	7.0 (4.0—9.0)	7.0 (5.0—13.0)	9.0 (2.0—11.0)	9.0 (7.0—12.0)	0.64	0.54	0.33
Number of unpleasant life events	4.0 (2.0—4.0)	5.0 (3.0—7.0)	4.0 (2.0—7.0)	5.0 (1.0—7.0)	2.0 (0.0—6.0)	5.5 (3.0—7.0)	0.36	0.84	0.17
Number of pleasant life events	3.0 (3.0—5.0)	4.5 (2.0—9.0)	2.0 (1.0—5.0)	3.0 (2.0—4.0)	2.0 (1.0—6.0)	3.0 (2.0—5.0)	0.69	0.60	0.86
Amount of total unpleasantness	11.0 (9.0—16.0)	15.6 (6.0—20.0)	11.0 (8.0—13.0)	14.0 (5.0—21.0)	6.0 (1.0—22.0)	17.0 (8.0—24.0)	0.89	0.74	0.22
Amount of total pleasantness	14.0 (9.0—18.0)	15.0 (6.0—21.0)	7.0 (5.0—17.0)	9.0 (5.0—16.0)	8.0 (2.0—20.0)	12.0 (7.1—16.0)	0.85	0.99	0.78
Daily hassles									
Total number of daily hassles	24.0 (14.0—26.0)	22.5 (14.0—32.0)	21.0 (15.0—26.5)	20.0 (16.0—37.0)	16.0 (12.0—18.0)	24.0 (12.0—37.0)	0.57	0.59	0.33
Intensity per hassle	1.5 (1.4—1.8)	1.3 (0.9—1.7)	1.5 (1.4—1.6)	1.4 (0.9—1.7)	1.2 (1.1—1.6)	1.4 (1.1—1.7)	0.08	0.52	0.92
Total intensity of all hassles	29.0 (14.0—50.0)	29.0 (12.0—43.0)	31.5 (20.1—42.0)	29.0 (20.0—56.0)	19.0 (14.0—32.0)	32.5 (17.0—52.0)	0.77	0.87	0.38
Hypothyroism study B2									
Positive and negative affect schedule scale (tendency to report)									
Positive feelings	39.0 (36.0—43.0)	38.0 (35.0—41.0)	35.0 (31.0—40.0)	39.0 (34.0—42.0)	37.0 (34.0—41.0)	38.0 (32.0—42.0)	0.21	0.10	0.92
Negative feelings	21.5 (19.0—27.0)	23.0 (18.5—28.5)	20.0 (15.0—26.0)	22.0 (17.0—26.5)	17.0 (14.0—21.0)	23.0 (16.0—28.5)	0.51	0.40	0.01
Recent life events									
Total number	7.5 (4.0—15.0)	10.5 (6.0—14.5)	8.5 (4.0—12.0)	8.0 (6.0—11.0)	9.0 (6.0—12.0)	7.5 (4.5—12.5)	0.13	0.66	0.23
Number of unpleasant life events	2.0 (1.0—6.0)	4.0 (2.0—6.0)	3.0 (2.0—6.1)	4.0 (1.5—5.5)	3.0 (1.0—5.0)	3.0 (1.0—5.0)	0.17	0.68	0.97
Number of pleasant life events	3.0 (1.0—6.0)	4.0 (3.0—6.5)	3.0 (1.0—6.0)	3.5 (2.0—6.0)	5.1 (3.0—7.0)	3.5 (2.0—5.0)	0.11	0.52	0.05
Amount of total unpleasantness	8.0 (3.0—19.2)	13.5 (8.0—21.5)	10.0 (4.0—20.2)	12.0 (7.5—19.5)	9.0 (4.0—17.7)	10.5 (5.0—18.0)	0.04	0.30	0.70
Amount of total pleasantness	12.5 (5.0—25.0)	14.5 (11.0—25.0)	11.5 (4.0—22.0)	13.0 (8.0—21.0)	16.7 (12.0—23.5)	11.5 (7.0—20.0)	0.13	0.43	0.05
Daily hassles									
Total number of daily hassles	18.0 (16.0—28.0)	23.5 (17.0—33.0)	20.0 (13.0—29.0)	23.0 (16.0—32.0)	19.5 (12.0—33.0)	22.5 (14.0—34.0)	0.09	0.24	0.54
Intensity per hassle	1.2 (1.0—1.4)	1.3 (1.0—1.6)	1.4 (1.1—1.7)	1.3 (1.0—1.7)	1.2 (1.0—1.6)	1.4 (1.1—1.6)	0.35	0.47	0.35
Total intensity of all hassles	23.0 (15.0—34.0)	27.0 (18.5—46.0)	24.0 (16.0—47.0)	28.0 (19.0—44.0)	24.5 (11.0—41.5)	28.0 (17.0—51.0)	0.10	0.78	0.34

Data are given as median with interquartile range between parentheses. *p*-Value of cases versus controls. Time of event: visit when diagnosis of overt hyper- or hypothyroidism was made or confirmed. PANAS: positive and negative affect schedule.

We observed no differences in any of the three stress questionnaires between hyperthyroid cases and controls at baseline, at time one year before occurrence of the event and at time of the occurrence of the event (Table 3).

At study entrance and at the year prior the event, we did not find any differences between hypothyroid cases and controls in recent life events, daily hassles, or affect scales (Table 3) except a lower amount of total unpleasantness in the hypothyroid cases than in controls at baseline. At the time of event, hypothyroid cases compared to controls reported significantly less frequently negative feelings at the time when diagnosis was made than controls. No other significant differences were found.

We also tested if AITD was more prevalent in subjects who were exposed to severe stress and for that reason we analyzed the data in order to uncover possible differences in the extreme ends of any of the stress measurements for both study A and study B. We found that the proportion of subjects in whom measurements were above the 80th percentile did not differ between cases and controls. When we used the quadratics of the stress measurements, the results did not change either.

5. Discussion

The aim of our case–control studies nested in the observational Amsterdam AITD cohort study (Strieder et al., 2008) was to evaluate in a prospective manner the involvement of stress in both the early stages (when thyroid antibodies develop but thyroid function is still normal) and late stages (when overt thyroid dysfunction emerges) of the natural course of AITD. The observed differences between cases and controls in both studies were either nonexistent or of marginal significance at study entrance and during follow-up, with the exception of a lower frequency of negative feelings reported at the time of the occurrence of hypothyroidism.

The thyroid autoimmune process takes years (Effraimidis et al., 2011), resulting in many cases in overt autoimmune hypo- or hyperthyroidism. All studies in the past on the relationship of stress and overt autoimmune hypo- or hyperthyroidism, measured stress by questionnaires at the time of the diagnosis. This approach raises the question of distinguishing between cause and effect, as the diseases themselves might generate stress in a patient. If stress would play a provocative role in the early stages of AITD, one would expect an association of stress and the occurrence of TPO-Ab, as the occurrence of TPO-Ab is one of the earliest events in AITD. Our results do not indicate such an association. These findings are in an agreement with our previous longitudinal cross sectional study (Strieder et al., 2005) in which no association was found between stressful life events, daily hassles and mood and the presence of anti-TPO antibodies in euthyroid women.

We observed no increase in stress between hypothyroid cases and controls at baseline, in the year preceding the event or at the time of event in recently life events and daily hassles. Only two papers in the past reported on the association between stress and autoimmune hypothyroidism. Both papers failed to find any association between stress and autoimmune hypothyroidism. In one study (Martin-du Pan, 1998) the triggering role of stress was assessed in 95 patients with Hashimoto's thyroiditis and 97 patients with benign thyroid nodules used as controls. Stress did not have any triggering role in Hashimoto's thyroiditis. Another study (Oretti et al., 2003) evaluated the association between stress and the occurrence of postpartum thyroiditis. There was no excess of life events in women who developed postpartum thyroiditis with no difference in the number of life events between antibody positive and antibody negative women. In agreement with these studies, our study also did not observe any differences between hypothyroid cases and controls. We only observed significant difference between hypothyroid cases and their respective controls on the PANAS negative affect scale, indicating a lower tendency to report negative feelings in cases at the time of event. If anything, we observed effects in the direction opposite to our expectation: we found that that hypothyroid cases, compared to their respective controls scored higher at the time of diagnosis on the PANAS negative affect scale, indicating a lower tendency to report negative feelings in cases. Moreover we found that at the time of diagnosis they also reported to have experienced more pleasant events and a higher total pleasantness of these events in the preceding 12 months.

We also did not detect any causal relationship between stressful life events and Graves' hyperthyroidism. Our findings are in contrast with several case–control studies in the past which have reported on a positive relationship between stress and the development of Graves' hyperthyroidism (Chopra et al., 1977; Winsa et al., 1991; Sonino et al., 1993; Kung, 1995; Chiovato and Pinchera, 1996; Radosavljević et al., 1996; Yoshiuchi et al., 1998; Dayan, 2001). All studies were retrospective in nature and were influenced of recall bias as Graves' disease patients were asked to report on stressful life events either at the time they were still hyperthyroid or even later when euthyroidism was restored. Furthermore, it could not be ruled out that negative, stressful life events in the year preceding the onset of Graves' hyperthyroidism were not the cause but the consequence of thyrotoxicosis. This issue was addressed to a certain extent in a study which reported an increase in negative life events in Graves' disease patients compared with non-autoimmune hyperthyroid subjects (Matos-Santos et al., 2001).

A weakness of our study is the limited number of subjects who converted from euthyroidism to overt hypothyroidism (N = 38) or hyperthyroidism (N = 13). It should be pointed out that cases in study B with thyroid antibodies at baseline are already at substantial higher risk of developing overt hypo- or hyperthyroidism, independently of stressful situations. A larger number of cases and a longer follow-up period are required to more accurately outline the role of stress. This limitation is in our view well balanced by the strengths of our study. Its prospective nature guarantees more solid evidence than obtained from cross-sectional studies. Moreover, in the nested case–control study a perfect match existed between both groups with respect to age and duration of follow-up. Higher age and longer exposure time both increase the likelihood of developing thyroid antibodies, constituting possible bias. The matching procedure effectively excluded both bias.

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Conflict of interest

All authors declare that they have no conflicts of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.psyneuen.2011.12.009.

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