CLINICAL STUDY

Sympatho-vagal control of heart rate variability in patients treated with suppressive doses of L-thyroxine for thyroid cancer

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Abstract

Objective: This study aimed to analyze the autonomic control of heart rate variability (HRV) in subjects receiving chronic L-thyroxine (L-T4) treatment after total thyroidectomy and ^{131}I therapy for differentiated thyroid carcinoma.

Methods: Blood pressure (BP) and sympatho-vagal activity (evaluated by power spectral analysis (PSA) of time-domain parameters of HRV) were studied in clinostatism and after orthostatism in 24 healthy controls, and in 12 patients taking L-T4 (125–200 $\mu\text{g/day}$) to maintain serum TSH levels at $<0.01 \mu\text{IU/ml}$. The study of HRV by PSA is a non-invasive method of analyzing sympatho-vagal control of HRV by quantifying high-frequency (HF) (0.15–0.4 Hz) and low-frequency (LF) (0.04–0.15 Hz) powers.

Results: Patients on L-T4 treatment had undetectable TSH levels, serum free T4 (fT4) above the normal range or at the upper limit in one case, and normal free tri-iodothyronine (fT3) levels. Heart rate and R–R intervals were not different in the two groups, both in clinostatism and in orthostatism. Systolic and mean BP were higher in patients than in controls and were inversely correlated with actual serum fT4 levels. During clinostatism, thyroid patients showed significantly lower LF power ($P = 0.035$), LF/(LF + HF) ($P = 0.008$) and LF/HF ($P = 0.01$) than controls. When patients moved from lying to standing, there was a significantly different decrease in orthostatic LF power ($P = 0.001$), LF/(LF + HF) ($P = 0.044$) and LF/HF ($P = 0.047$) versus controls.

Conclusions: Changes in autonomic control of HRV, characterized by decreased sympathetic activity and impaired sympatho-vagal balance with preserved vagal tone, are detectable in patients with hyperthyroxinemia due to suppressive L-T4 therapy and increased systolic and mean, but not diastolic, BP.

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Introduction

Thyroid hormones affect cardiac function through their direct effects on the heart or by influencing sympatho-vagal tone, with hyperthyroid patients presenting signs and symptoms mimicking a sympathetic overactivity (1–3).

Overt hyperthyroidism is known to alter both heart performance and morphology (1–7). However, myocardial changes and dysfunction (faster heart rate, increased left ventricular mass and enhanced function, altered diastolic filling), more frequent occurrence of atrial fibrillation and impaired cardiac autonomic control have also been reported in patients with subclinical hyperthyroidism (7–11). This condition is characterized by lowered or undetectable thyroid-stimulating hormone (TSH) levels and free thyroid hormone concentrations persistently within the

normal range (12). Cardiovascular alterations, although less intensive, have also been detected when doses of L-thyroxine (L-T4) inhibiting TSH secretion were administered (6, 7, 13–18).

Despite clinical evidence for a sympathetic overactivity, a great deal of uncertainty persists as to the interaction between the autonomic system and thyroid hormones in the control of heart rate and function. The study by power spectral analysis (PSA) of electrocardiographic (ECG) R–R intervals, and of acute physiological adaptative response to postural changes, is a reliable, sensitive and non-invasive tool for measuring sympatho-vagal balance in humans (19, 20).

Changes in autonomic control of heart rate variability (HRV) have been demonstrated by PSA in overt hyperthyroidism and to a lesser extent in patients with spontaneous subclinical hyperthyroidism, but some discrepancies exist among the various reports

due to clinical differences in patients studied and in methodological approaches (21–26).

In this study, we analyzed HRV by PSA in a group of subjects chronically treated with L-T4 to inhibit TSH secretion after thyroid ablation by surgery followed by radioactive ^{131}I (RAI) therapy for differentiated thyroid carcinoma (DTC). Suppression of TSH secretion by exogenous L-T4 administration involves the use of hormone dosages that induce a supra-physiological increase in circulating levels of free T4 (fT4), converted to tri-iodothyronine (T3) by peripheral deiodinases. Thus, this condition appears to be different both from overt hyperthyroidism and from spontaneous subclinical hyperthyroidism, in which thyroid hormone levels in serum are normal by definition.

Subjects and methods

Subjects

Twelve patients (2 male, 10 female), aged between 36 and 69 years (mean age $\pm$ s.d., 56.8 ± 9.0 years), were enrolled in this study. They underwent total thyroidectomy followed by RAI for DTC of papillary or follicular type and were treated with L-T4 attaining suppression of TSH levels between 4 months and 3 years before the study. They attended routine follow-up for DTC and gave informed and written consent to the PSA study according to the Helsinki declaration.

Patients were treated with L-T4 at doses ranging between 125 and 200 $\mu\text{g/day}$ orally ($1.5\text{--}2.2 \mu\text{g/kg}$ body weight) and presented persistently suppressed TSH levels ($<0.005 \mu\text{IU/ml}$). Patients took their current doses of L-T4 at 0700 h and TSH, free fractions of thyroid hormones and thyroglobulin (Tg) serum levels were assayed between 0800 and 0900 h on the same days before the autonomic tests. All patients were free from disease recurrence and their Tg levels were below the lowest normal range limit ($<0.5 \text{ pg/ml}$).

Cardiovascular data in patients were compared with those of 24 sex- (4 male, 20 female) and age-matched healthy controls, 38–67 years old (mean age $\pm$ s.d., 54.3 ± 15.6 years), with normal thyroid and heart function. Patients and controls did not take drugs interfering with cardiovascular function. No smoking or consumption of coffee, tea or other stimulating substances were allowed during the 6 h before the study.

Assay methods

Serum levels of TSH, fT4 and fT3 were measured using ultrasensitive chemiluminescence immunoassays (Roche). The third-generation ultrasensitive TSH method was standardized against the 2nd IRP WHO Reference Standard 80/558; it had a lower detection limit of $0.005 \mu\text{IU/ml}$ and sensitivity of $0.01 \mu\text{IU/ml}$.

A one-step immunoradiometric method (Techno Genetics, Milan, Italy) was used for Tg assay.

HRV analysis

An ECG recording of R–R intervals was made at 1000 h in a quiet dedicated room, 1 h at least after venipuncture for routine hormonal evaluation. Subjects were placed supine on the tilting table and instructed to relax, stay awake, breathe regularly, and not to speak. After supine resting for about 10 min to stabilize blood pressure and heart rate (HR), clinostatic R–R intervals were acquired. The recording was repeated immediately thereafter with the move from lying to standing achieved by a 30-s passive shift using a mechanically driven tilt table.

For both clinostatic and orthostatic posture, ECG was continuously recorded for 330 s by means of an ECG connected to a personal computer equipped with software that sampled the analogical signal at about 200 Hz using an analog–digital converter. Every R–R interval was measured in milliseconds and memorized as a tachogram (R–R interval duration vs number of progressive cardiac beats). Two series of data corresponding to clinostatic (tachogram A) and orthostatic (tachogram B) R–R intervals were analyzed in all subjects by a parametric method, based on the autoregressive model for the quantification of HRV signal (27).

The main power densities in the high-frequency (HF) ($0.15\text{--}0.4 \text{ Hz}$) and low-frequency (LF) ($0.04\text{--}0.15 \text{ Hz}$) bands were identified for each density spectrum. HF power is thought to be a marker of the modulation of vagal tone, while LF power is mainly a measure of the modulation of vagal and sympathetic tone by baroreflex activity (28, 29). In addition, both $\text{LF}/(\text{LF} + \text{HF})$ and LF/HF ratios were calculated. The first ratio is an index of sympathetic power with respect to the sum of LF and HF absolute values, while LF/HF is to be considered as an index of sympatho-vagal balance in the frequency domain (28).

Mean R–R intervals (ms) and corresponding HR (beats/min) were calculated on the 330s tachogram series obtained in clinostatism and orthostatism.

Blood pressure (BP) was preliminarily measured in both arms by an automatic pressurometer (AMI System 7100) to exclude inter-arm differences. Then, mean values of three consecutive systolic, mean and diastolic BP (SBP, MBP and DBP respectively) values in clinostatism were calculated; BP was again measured after 1 min of orthostatism.

Statistical analysis

All results are given as the mean values $\pm$ s.d. Data were analyzed using the Prism 4.0 software (GraphPad, San Diego, CA, USA) and preliminarily processed by the Kolmogorov–Smirnov test to assess their normality and skewness. Because of the non-parametric distribution of

some data and indices of autonomic function, the Mann–Whitney *U* test for unpaired data was applied for comparisons of data between patients and healthy controls. Statistical differences in PSA data observed in clinostatism and orthostatism in each of the two groups were analyzed by the Wilcoxon test. Correlations between thyroid hormone levels and BP, HR and PSA data were analyzed by Pearson's *r* correlation test. The level of significance was set at $P < 0.05$.

Results

Endocrine data

Serum TSH levels were undetectable ($<0.005 \mu\text{IU/ml}$) in all patients (normal range, $0.3\text{--}4.2 \mu\text{IU/ml}$). Serum fT_4 levels were between 21.9 and 28.8 pmol/l and exceeded the normal range in all but one patient (mean levels $\pm$ s.d., $24.5 \pm 2.3 \text{ pmol/l}$; normal range, $12.0\text{--}22.0 \text{ pmol/l}$). The fT_3 levels were normal in all patients, ranging between 3.5 and 6.1 pmol/l (mean $\pm$ s.d., $5.1 \pm 0.7 \text{ pmol/l}$; normal range, $3.5\text{--}6.8 \text{ pmol/l}$).

BP data and PSA analysis of HRV

SBP and MBP, but not DBP, were slightly higher in DTC patients than in healthy controls (Table 1).

No significant difference was observed between R–R interval duration and HR, neither in patients nor in controls, during clinostatism and orthostatism (Table 2). Moving from lying to standing significantly shortened R–R intervals and accelerated HR in controls. A similar effect on HR was obtained in thyroid patients, who showed changes in R–R intervals after orthostatism that were at the limit of statistical significance (Table 2).

PSA data of HRV during clinostatism and orthostatism in the two groups are reported in Figs 1 and 2. Lying DTC patients presented significantly lowered LF power, but no difference was observed in HF power between the two groups (Fig. 1). Moreover, DTC patients had significantly lowered LF/(LF + HF) and LF/HF ratios indicating an alteration of sympathetic control of HRT during clinostatism (Fig. 2).

Moving from lying to standing did not modify LF power in patients, while a physiological

(but not significant) increase was observed in controls. Postural change induced a significant fall in HF power in both groups of subjects, along with an increase in LF/(LF + HF) and LF/HF ratios when compared with clinostatic data (Figs 1 and 2).

Changing from lying to standing amplified the difference existing between LF powers in patients and in controls, orthostatic LF/(LF + HF) and LF/HF remaining significantly lowered in the first group. In addition, no statistical difference was observed in this instance between orthostatic HF values in patients and controls.

Clinostatic SBP ($r = -0.6200$; $P = 0.0315$) and MBP ($r = -0.6316$; $P = 0.0276$) values were inversely correlated with serum fT_4 levels in DTC patients. No other significant correlations were observed between cardiovascular parameters and thyroid hormone levels, L-T4 dosages or L-T4 treatment duration.

Discussion

In patients treated with TSH-suppressive doses of L-T4 for DTC, we did not find significant differences in R–R intervals and HR during clinostatism and after moving from lying to standing, in comparison with healthy controls. This finding agrees with previous observations on R–R intervals in resting subjects with spontaneous subclinical hyperthyroidism (25). Increased HR (9) and reduced average R–R intervals (24) were instead found by an ECG 24-h Holter recording in free-moving subclinical hyperthyroid subjects. In both groups we studied, HR was enhanced by standing up, but rapid posture changes increased HR in controls more than in patients. This behaviour was consistent with an impaired sympathetic activity expressed by lowered LF but unchanged HF power emerging from PSA of HRV in patients.

LF power is an index of both sympathetic and vagal modulation by baroreceptor activity, mainly reflecting the effects of the former (29); HF power is a marker of vagal activity modulation by the frequency and depth of ventilation (30, 31).

The impairment of LF baroreflex-mediated autonomic activity was emphasized by lowered LF/(LF + HF) and LF/HF ratios. These indices are expressive of the balance of autonomic control on HRV and better define shifts from vagal to sympathetic dominance,

Table 1 SBP, MBP and DBP values in clinostatism and 1 min after orthostatism in 12 patients with DTC on chronic L-T4 treatment, and in 24 healthy controls. Statistical analysis: Mann–Whitney *U* test for unpaired data.

	Clinostatism			Orthostatism		
	SBP	MBP	DBP	SBP	MBP	DBP
DTC patients	135.5 ± 11.6	114.5 ± 9.5	72.6 ± 8.1	137.0 ± 12.0	118.2 ± 10.1	80.6 ± 9.3
Healthy controls	125.9 ± 9.4	108.1 ± 7.3	72.7 ± 8.6	123.6 ± 7.6	108.1 ± 6.7	77.0 ± 9.0
<i>P</i>	0.011	0.033	0.906	0.015	0.014	0.289

Table 2 R–R interval duration and heart rate (HR) during clinostatism and orthostatism in 12 patients with DTC treated with suppressive doses of L-T4 and in 24 healthy controls. Statistical analysis: Mann–Whitney *U* test for unpaired data and Wilcoxon test for paired data.

	R–R interval (ms)			HR (beats/min)		
	Clinostatism	Orthostatism	<i>P</i>	Clinostatism	Orthostatism	<i>P</i>
DTC patients	892.4±135.3	840.7±229.3	0.064	68.7±10.8	75.2±15.7	0.034
Healthy controls	837.8±95.8	756.0±114.8	0.0002	72.4±7.6	81.1±12.1	0.0002
<i>P</i>	0.347	0.411		0.347	0.411	

their lowering suggesting a sympathetic withdrawal (19, 28).

Cacciatori *et al.* (21) and Burggraaf *et al.* (23) have found an impairment of HF parasympathetic activity in hyperthyroid patients, with some differences concerning the decrease in LF power which was unchanged or only slightly reduced.

After an ECG 24-h Holter recording, Petretta *et al.* (24) reported a lowered LF power and a not significant decrease in LF/HF in patients with endogenous sub-clinical hyperthyroidism, but a cardiac parasympathetic withdrawal with an increase of LH/HF was present in those with overt hyperthyroidism (24). The finding has recently been confirmed in a very large number of hyperthyroid patients before treatment (26).

Goichot *et al.* (25) reported that, during a 5-min observation, there were no significant differences in LF power and LF/(LF + HF) ratios between healthy controls and patients with either overt or subclinical hyperthyroidism. In this series, only HF was significantly decreased in overt hyperthyroidism, but not in subclinical hyperthyroidism.

These data indicate that a decrease in vagal activity rather than an activation of the sympathetic tone characterizes the autonomic control of heart function in overt hyperthyroidism (21–26). Thus, reduced cardiac sympathetic activity at the early stage of hyperthyroidism or hyperthyroxinemia, as well as

during subclinical hyperthyroidism, apparently differs from the vagal impairment and clinically evident sympathetic overactivity in the overt stages of the disease.

An inverse correlation was found between fT4 levels and SBP and MBP in our patients. A negative correlation between thyroid hormone concentrations in serum and sympathetic nerve activity in muscle was also reported by Matsukawa *et al.* (32). Moreover, motor vagal neuron excitability in response to carotid-deal baroreceptor activation was found to be impaired in hyperthyroidism (33) and resting HR appeared mainly affected by a marked reduction in the efferent activity of the parasympathetic component (33, 34).

Moving from lying to standing involves a complex adaptative response to maintain BP and brain perfusion. This physiological effect mainly consists of a sympathetic activation, which increases HR and output, combined with an inhibition of the vagal tone and increased LF/HF (28, 35).

After postural variation in patients, we observed an inadequate response of LF power, which even decreased with respect to the values recorded in clinostatism (−9.1% in patients vs +19.6% in controls). A more evident fall was observed in both groups in the HF power (−66.5% in patients vs −42.3% in controls).

Our patients had SBP and MBP slightly but significantly higher than those in healthy controls, while diastolic BP was similar in the two groups. The chronic inhibition of TSH secretion by increased thyroid

Figure 1 Low-frequency (LF) and high-frequency (HF) PSA components during clinostatism and orthostatism in 12 patients with DTC treated with suppressive doses of L-T4 and in 24 healthy controls. Statistical analysis: Mann–Whitney *U* test for differences between patients and healthy controls (*P* values indicated by asterisks), and Wilcoxon test for the comparison between clinostatic and orthostatic data within groups (*P* values shown above the bars).

Figure 2 LF/(LF + HF) and LF/HF ratios in clinostatism and orthostatism in 12 patients with DTC treated with suppressive doses of L-T4 and in 24 healthy controls. For statistical analysis see legend to Fig. 1.

hormones is combined with greater left ventricular mass and enhanced inotropic response and cardiac output (3, 5, 10). These changes in myocardial morphology and function, as well as slightly increased SBP, might determine a baroreflex-mediated impairment in sympathetic outflow and activity as observed in early experimental hyperthyroidism (36). Moreover, thyroid hormones may act directly on central nervous system areas that integrate autonomic function (33).

In conclusion, an impairment in sympatho-vagal balance, consisting of a sympathetic failure and altered autonomic mechanisms in response to the change from lying to standing, occurred in patients on long-term treatment with suppressive doses of L-T4 for DTC. These changes possibly depend on a mild increase in SBP, which might modify sympathetic outflow acting on baroreceptors regulating HRV.

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