Clinical aspects of endogenous hypothyroidism and subclinical hyperthyroidism in patients with differentiated thyroid carcinoma

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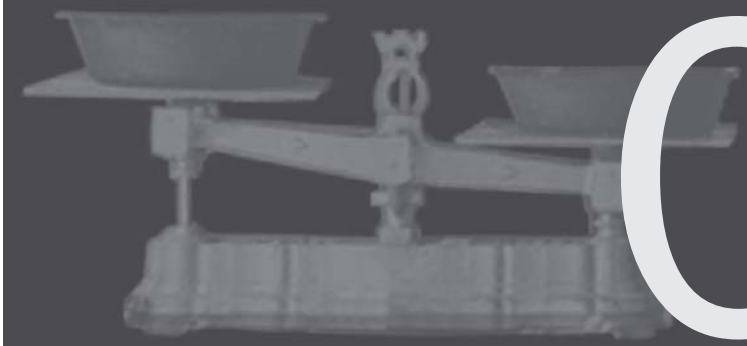

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Autonomic nervous system function in chronic exogenous subclinical thyrotoxicosis and the effect of restoring euthyroidism

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Abstract

Context: Knowledge on the relationship between the autonomic nervous system and subclinical hyperthyroidism is mainly based upon cross-sectional studies in heterogeneous patient populations and the effect of restoration to euthyroidism in subclinical hyperthyroidism has not been studied.
Objective: We investigated the long-term effects of exogenous subclinical hyperthyroidism on the autonomic nerves system and the potential effects of restoration of euthyroidism.
Design: Prospective single blinded, placebo-controlled, randomized trial.
Setting: University Hospital.
Patients: 25 patients who were on >10 years of TSH suppressive therapy after thyroidectomy.
Intervention: Patients were studied at baseline and subsequently randomized to a 6-months thyroid hormone substitution regimen to obtain either euthyroidism or maintenance of the subclinical hyperthyroid state.
Main outcome measures: Urinary excretion of catecholamines and heart rate variability were measured. Baseline data of the subclinical hyperthyroidism patients were compared to data obtained in patients with hyperthyroidism and controls.
Results: Urinary excretion of NE and VMA was higher in the subclinical hyperthyroidism patients compared to controls and lower compared to patients with overt hyperthyroidism. Heart rate variability was lower in patients with hyperthyroidism, intermediate in subclinical hyperthyroidism patients and highest in the healthy controls. No differences were observed after restoration of euthyroidism.
Conclusions: Long term exogenous subclinical hyperthyroidism has effects on the autonomic nerves system measured by heart rate variability and urinary catecholamine excretion. No differences were observed after restoration to euthyroidism. This may indicate occurrence of irreversible changes or adaptation during long-term exposure to excess thyroid hormone that are not remedied by 6 months of euthyroidism.

Introduction

Overt hyperthyroidism has profound effects on the heart, including tachycardia and/or arrhythmias, increased systolic pressure, increased systolic function, left ventricular hypertrophy and diastolic dysfunction (1,2,3). These effects are thought to be the result of direct effects of thyroid hormone on the cardiovascular system and the interaction of thyroid hormones with the sympathetic nervous system (2,4). This interaction has been shown to result from a sympathovagal imbalance, characterized by increased sympathetic activity in the presence of diminished vagal tone, which coincides with increased urinary excretion of catecholamines (5,6,7). Hence, the current consensus is that manifestations of altered autonomic nervous system function play a role in the pathophysiology and clinical presentation of thyrotoxicosis.

For subclinical hyperthyroidism, defined as low serum thyroid stimulating hormone (TSH) concentrations despite normal free thyroxin (FT4) and tri-iodothyronine (T3) concentrations, cardiovascular effects may also occur, but these are less well known and seemingly less severe. The most consistent findings include increased heart rate, supraventricular arrhythmias and abnormalities of LV morphology and function (8,2,9,10). Altered autonomic nervous system function in subclinical hyperthyroidism is also less well defined. Petretta et. al. (9), Goichot et. al. (11) and Portella et. al. (12), using measures of heart rate variability , found evidence that in patients with endogenous subclinical hyperthyroidism a reduction of cardiac parasympathetic control is present and this is supported by findings on heart rate turbulence by Osman *et al* (13). However, in the study of Goichot (11) there were no differences in the heart rate variability measure (the ratio of low frequency power over high frequency power: LF/HF) that is commonly used to characterize the balance between vagal and sympathetic influences in these patients. In addition, it seems that the most prominent differences between patients with (subclinical) hyperthyroidism and controls were present during a challenge of the autonomic nervous system. Apart from this, the interpretation of these findings is difficult as studies on the role of the possibly altered autonomic nervous system abnormalities and the cardiovascular consequences of subclinical hyperthyroidism are complicated by several factors. First, subclinical hyperthyroidism is a heterogeneous clinical syndrome with many possible etiologies with as sole common denominator the (biochemical) definition of low TSH and normal T3/T4 concentrations. Second, the duration and course of the underlying disease is often not known and therefore it cannot be excluded that the underlying disease itself, treatment with thyreostatic medication and use of β-blockers may have influenced cardiovascular parameters independent of serum thyroxin levels.

These considerations suggest that the most appropriate population to study the consequences of subclinical hyperthyroidism are patients treated for differentiated thyroid carcinoma (DTC) in whom, after thyroidectomy, continuous suppression of TSH occurs with individualized doses of levothyroxine (L-thyroxin). In these patients, subclinical hyperthyroidism is solely the result of exogenous L-thyroxin. We therefore performed a prospective, randomized, placebo-controlled study to assess autonomic nervous function in patients with DTC with longer than 10 years exogenous subclinical hyperthyroidism and to investigate whether restoration to euthyroidism affects autonomic nervous function. Autonomic nervous function was assessed using urinary catecholamine excretion, heart rate variability measurements during rest and by measuring the response in heart rate to a standardized mental stress test.

Subjects and methods

The ethics committee of Leiden University Medical Center (LUMC) approved the study protocol, and written informed consent was obtained from all subjects. The study was performed in compliance with the principles of the Declaration of Helsinki.

Subjects

Patients treated for DTC were recruited from the outpatient clinic of the Department of Endocrinology of the LUMC, a tertiary referral centre for DTC. Patients were included who had been diagnosed with DTC, and had received initial therapy consisting of total-thyroidectomy and radioiodine ablative therapy. Cure was documented by the absence of measurable serum thyroglobulin (Tg) during TSH stimulation as well as by a negative total-body scintigraphy with 4 mCi I-131. Patients had been on TSH suppressive therapy, defined as TSH levels below the lower reference values for normal serum levels of TSH (0.4 mU/L), for at least 10 years. The adequacy of this therapy was documented by yearly TSH measurements. Patients were excluded when they used medication affecting the sympathetic nervous system or when they were currently treated for or had experienced major cardiovascular events as uncontrolled hypertension or a myocardial infarction.

The study was a prospective, single-blinded randomized study of 6 months duration with 2 parallel groups. After inclusion, patients were randomized in a single-blinded fashion (patients were blinded) to a maintenance group or an intervention group. Only the treating physician prescribing the study medication was aware of the randomization. The other research staff involved in study-related activities was also blinded to treatment. In the maintenance group the existing TSH-suppressive therapy was continued (target TSH level <0.4 mU/L). In the intervention group it was attempted to reach a restoration of euthyroidism by decreasing the L-thyroxin dose target TSH levels within the normal reference range (0.4-4.8 mU/L)). This was achieved by replacing in all patients the standard L-thyroxin therapy in part by study medication according to an algorithm. Study medication consisted of either L-thyroxin 25 µg or identically looking placebo tablets. Serum TSH levels were checked every 6 weeks in every patient, and study medication was adjusted if necessary to obtain the target TSH levels.

Patients were compared to data obtained in patients with overt hyperthyroidism and healthy controls using similar methodology (5).

Before and after 6 months, identical assessments were performed. After an overnight fast, subjects were admitted to the clinical research unit, where the urine collected over the previous 48 hrs was handed in. After a medical history and physical examination, blood samples to assess thyroid hormone status were taken. At least 30 minutes after blood sampling, continuous ECG and blood pressure measurements were made while the subject was in supine position for at least 15 minutes. During this period the patients were acquainted with the test procedures that were about to follow. The measurements consisted of a 1-lead electrocardiogram (ECG) registration (recording 600 subsequent beats). The subjects were instructed to relax, to breathe regularly, not to speak and to stay awake. ECG signals were sampled at a rate of 500 Hz and the arterial pulse wave at a rate of 300 Hz. The signals were digitized using a customized laboratory interface (model 1401, Cambridge Electronic Design, Cambridge, UK), and analyzed with software supplied with the interface. Each registration was screened for artefacts and subsequently analyzed for heart rate variability parameters in the time domain: mean RR-interval (RR-int), the coefficient of variation (CV) of the successive RR-intervals (reflecting total variability), and the standard deviation of differences between adjacent R-R intervals (SDSD) reflecting “beat-to-beat” and, therefore, vagally mediated variability) as previously described (5) and according to the applicable guidelines (14).

The registrations were also analyzed for heart rate variability parameters in the frequency domain according to the same guidelines (14). Upon completion of the recording at rest, another 5-min recording was started during which the subjects were subjected to a mental stress test (15). During this test the subjects had to perform a standardized arithmetic test about which they had been instructed before. The registration made during this test was used to determine the percentage increase in heart rate from baseline.

Assays

Thyroid hormones, thyroid-stimulating hormone (TSH), and urinary creatinine and catecholamines concentrations were determined using standardized routine methodology at the clinical chemistry laboratories of the LUMC.

FT_4 was measured on an IMx (Abbott, Abbott Park, IL; intra-assay variability: 2.5-7.6%, interassay variability: 5.6-12.4%) at different levels). Total T_4 was determined on the TDx (Abbott; interassay CVs: 2.4-5.9%). Free tri-iodothyronine (FT3) was measured by RIABEAD (Abbott; interassay CVs of 2.0-4.4%). Serum TSH was determined with a Modular Analytics E-170 system (Roche Diagnostic Systems, Basle, Switzerland), interassay variability:0.88-10.66%). Reference values for FT4, FT3 and TSH are respectively 10-24 pmol/L, 2.5-5.4 pmol/L and 0.4-4.8 mU/L. Urinary norepinephrine (NE), dopamine (DOPA) and vanillylmandelic acid (VMA) were determined by routine HPLC methodology.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). For assessment of the treatment effect between groups, the variables were log-transformed to meet the requirements for analysis of variance. Subsequently the transformed data were analyzed using analysis of covariance (ANCOVA, SAS Proc MIXED) with the baseline value as co-variate. Treatment least square means were back-transformed resulting in geometric mean treatment estimates corrected for differences in the baseline values. Contrasts and 95% confidence intervals (95% CI) between treatments were back-transformed resulting in geometric mean ratios, which were subsequently translated into percentage increase of the therapy treatment relative to the maintenance treatment.

The data obtained at baseline in the subclinical hyperthyroidism patient cohort were compared with data obtained in patients with overt hyperthyroidism and healthy controls using ANOVA and unpaired Student's t-test assuming unequal variances. The latter data were obtained using similar methodology and were reported earlier by our group (5). These data were also used to perform a post-hoc power analysis (using power=80% and alpha=5%) to calculate the required sample size per group for detecting relevant changes in the study parameters. Relevant changes were defined as the change required for normalizing the values obtained in the subclinical hyperthyroidism patients to the values observed in healthy controls.

All analyses were performed using SAS software (V9.1.2, SAS Institute, Inc., Cary, NC, USA).

Results

Patient characteristics

Thirty-three patients who fulfilled all inclusion criteria were included in the study. Four patients left the study before randomization: 1 patient because of abdominal surgery, 2 patients withdrew consent because of the perceived burden of the study and 1 patient did not present at the randomization visit.

During the study 3 patients from the intervention group were withdrawn: 1 patient (at 12 weeks) because of fatigue, headache and diarrhea, a second patient left (at 6 weeks) because of pregnancy and the third patient was excluded because of apparent incompliance: despite lowering the thyroxin dose, serum FT4 levels increased throughout the study. Two patients in the maintenance group (persistent low TSH) were also excluded for apparent incompliance; TSH levels rose despite being in the TSH suppression group. Thus 25 subclinical hyperthyroidism patients completed the study, 12 patients in the intervention group and 13 patients in the maintenance group. A summary of the subject characteristics is given in table 1.

Table 1. Characteristics of the study population

	Maintenance group (persistent TSH suppression) (n=13)	Intervention group (restoration euthyroidism) (n=12)	Control group (n=15)	Hyperthyroidism group (n=15)
Gender (F : M)	9 : 4	8 : 4	14:1	14:1
Age (yr)	49 ± 7.2 (36-64)	51 ± 10.5 (36-67)	40 ± 10.3 (21 - 56)	39 ± 9.7 (21-56)
Weight (kg)	77 ± 11.5 (60-103)	74 ± 9.8 (58-91)	68 ± 13.9 (47-97)	65 ± 11.9 (44-83)

Data are presented as mean ± SD (range).

L-thyroxin dose and thyroid hormones

The mean ± SD L-thyroxin dose in the maintenance group was 164 ± 34 µg/day before randomisation and remained virtually unchanged at 173 ± 28 µg/day at the second assessment. In the intervention group, the L-thyroxin dose was reduced from 185 ± 39 µg/day to 129 ± 37 µg/day in order to restore euthyroidism. The thyroid hormone levels are summarized in table 2. Thyroid hormone concentrations were not different between the groups at baseline. Particularly, the range of TSH concentrations was 0.003 - 0.339 mU/L in the maintenance group and 0.003 - 0.302 mU/L in the intervention group. At the end of the study, TSH concentrations were higher in the intervention group (range: 0.218 - 6.09 mU/L), while these remained virtually unchanged in the maintenance group (0.005- 0.210 mU/L). Eight patients in the intervention group became euthyroid 2 months after thyroxin dosage reduction, one patient 3 months after thyroxin dosage reduction and 2 patients 4 months after thyroxin dosage reduction. In these latter patients, FT4 levels decreased significantly every month, whereas TSH levels stayed behind. FT3 concentrations decreased by 42% (95% CI: 19-69%) and FT4 concentrations by 29% (95% CI: 12-48%) in the intervention group compared to the maintenance group. One patient in the intervention group had a persistently low TSH (<0.4 mU/L) for the duration of the study. After 6 months, this patient had a TSH of 0.218 mU/L, however, her baseline TSH was 0.0025 mU/L. Since her FT4 decreased from 21 tot 14 pmol/L, we considered the intervention in this patient successful, although her TSH at 6 months was still below 0.4 mU/L. Two patients in the intervention group had a TSH>4.8 mU/L (5.80 and 6.09 mU/L) at the end of the study. FT4 levels were 18.8 and 26.7 mmol/L.

Urinary catecholamine excretion

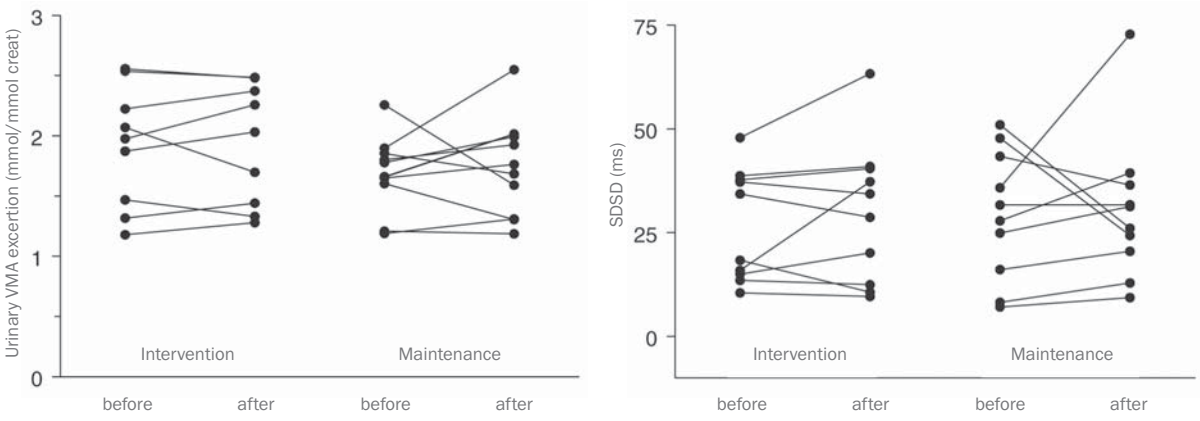
The urinary excretion of NE, DOPA and VMA (normalized for creatinine) is summarized in table 2. In order to illustrate the effects of longitudinal follow-up and the intervention, the urinary excretion of VMA for both groups is depicted in figure 1. This shows that in general the urinary excretion is remarkably stable during a 6-month follow-up period without treatment. Restoration to euthyroidism did not result in significant reductions in catecholamine excretion.

Table 2. Summary of thyroid hormone status and urinary catecholamine excretion normalized for creatinine excretion

	Maintenance group (persistent TSH suppression)		Intervention group (restoration euthyroidism)		Between group comparison
	before	after	before	after	p-value
TSH (mU/L)	0.08 ± 0.10	0.05 ± 0.02	0.10 ± 0.11	2.97 ± 2.30	p <0.001
FT3 (pmol/L)	3.4 ± 0.6	3.6 ± 0.6	3.6 ± 0.4	2.6 ± 0.6	p <0.001
FT4 (pmol/L)	22.8 ± 4.3	23.6 ± 1.0	22.6 ± 3.9	18.5 ± 4.1	p <0.001
Norepinephrine*	21.0 ± 6.5	23.6 ± 7.6	26.1 ± 7.4	24.7± 6.6	p =0.40
Dopamine*	0.17 ± 0.07	0.13 ± 0.04	0.15 ± 0.07	0.10 ± 0.04	p =0.40
VMA*	1.76 ± 0.35	1.72 ± 0.42	1.97 ± 0.48	2.03 ± 0.48	p =0.83

Data are presented as Mean ± SD. In the last column the p-value for the difference between the groups is given (using ANCOVA with baseline as co-variate).
TSH: thyroid stimulating hormone; FT3: free tri-iodothyronine; FT4: free thyroxin (FT4); VMA: vanillylmandelic acid.
** expressed as mmol/mmol creatinine*

Figure 1 Scatter plots showing the urinary vanillylmandelic acid excretion (VMA; left) and the SDSD measurements (right) illustrating that, except for the occasional outlier, both parameters were remarkable stable for the patients whether treated or not.



Autonomic nervous system function tests

The RR-interval in the intervention group increased from a mean value of 899 ± 135 milliseconds (ms) to 956 ± 135 ms (p =0.04). In the patients in whom thyroid suppression was continued, the RR-interval before the study was 849 ± 29 ms and this was not changed at the end of the study (869 ± 25 ms). Between the groups the change in RR-interval was not significantly different (6.7%; 95%CI: -0.5, 14.4%; p= 0.07). Both the time domain parameter reflecting the overall variability (CV) and the parameter reflecting the vagal influence on heart rate (SDSD) remained unchanged both within and between the treatment groups. The difference between the groups in CV and SDSD at the end of the treatment period were 3.6 (95% CI: -18.9, 32.3%) and 9.8% (95%CI: -25.0, 60.8%) respectively. The measurements of the SDSD for both groups are also depicted in figure 1. The data in the frequency domain were also not different between the groups (data not shown). All data are summarized in table 3. The difference in the increase in heart rate observed during the mental stress test was 9.0% (95%CI: -37.4; 32.3%) between the groups.

Table 3. Heart rate variability parameters in the time domain.

	Maintenance group (persistent TSH suppression)		Intervention group (restoration euthyroidism)		Between group comparison
	before	after	before	after	p-value
RR-int (ms)	849 ± 29	869 ± 25	899 ± 135	956 ± 135*	0.068
CV RR-int (%)	4.7 ± 1.6	4.7 ± 1.7	4.6 ± 2.4	4.4 ± 1.4	0.765
SDSD (ms)	35.8 ± 3.5	42.5 ± 5.6	26.3± 12.5	35.5 ± 24.2	0.614
HR response (%)	23 ± 3	23 ± 3	28 ± 17	24 ± 16	0.606

Data is expressed as mean ± SD The last column shows the p-value for the difference between the groups (using ANCOVA with baseline as co-variate).

RR-int: RR-interval; CV RR-int: coefficient of variation in RR-interval; SDSD: standard deviation of differences between adjacent R-R intervals; HR response: increase in heart rate during a mental stress test.

**p=0.04 for difference between before and after treatment*

Comparison between patient groups and power calculation

First, it is of note that the groups were comparable (ranges in age and weight show great overlap) albeit there were minor differences, particularly in weight which is obviously not surprising as patient with overt hyperthyroidism tend to lose weight (table 1). There was a difference between the groups regarding gender distribution with a female predominance in the comparison groups. However, there are no indications that gender is an important determinant of autonomic nervous system function (16).

There was a slight difference in age between the groups, and it has been shown that increasing age is related to a decline in heart rate variability-related parameters (17). However, the difference in age between the groups was small and even overlapping, making it unlikely that this may have caused important differences in the heart rate variability presented here.

Table 4 summarizes the results obtained at baseline in the current study and the data obtained in patients with overt hyperthyroidism and healthy controls.

The ANOVA analysis showed that the urinary excretion of NE (p=0.03) and of VMA (p=0.003) differed between the groups. The analysis shows that urinary excretion of the catecholamines was lower in the healthy controls compared to patients with subclinical hyperthyroidism; the mean (95% CI) difference was 3.80 mmol/mmol creatinine (-0.46/+8.061; p=0.053) for NE excretion and 0.434 mmol/mmol creatinine (+0.194/+0.675; p<0.001) for VMA excretion. Comparing the patients with subclinical hyperthyroidism with patients with overt hyperthyroidism showed a difference for the NE excretion of 4.00 mmol/mmol creatinine (-1.83/+9.83; p=0.217) and a difference in VMA excretion of -0.139 mmol/mmol creatinine (-0.4/+0.1223; p=0.275).

Analysis of variance showed significant differences between the groups for the measures of heart rate variability; the p-value was <0.001 for the RR-interval, a p-value of 0.0018 was observed for the coefficient of variation and for the differences in SDSD the p-value was <0.001. Patients with hyperthyroidism had on average a lower RR-interval of 280 ms (+201/+359; p<0.0001) than the patients with subclinical hyperthyroidism. This was accompanied with lower measures of heart rate variability; the coefficient of variation was 1.50% (+0.30/+2.70; p= 0.0088) lower, and the SDSD was 22.14 ms lower (+8.95/+35.33; p=0.0002). Comparing the patients with subclinical hyperthyroidism to the healthy controls showed that the RR-interval was 33 ms lower (-48/+114; p= 0.402). The coefficient of variation in heart rate was 1.88% (-0.04/+3.72; p= 0.08) lower and the SDSD was 12.92

Table 4. Urinary catecholamine excretion (normalized for creatinine) and heart rate variability parameters.

	Urinary catecholamine excretion		Heart rate variability		
	NE (mmol/mmol creatinine)*	VMA# (mmol/mmol creatinine)	RR-interval& (ms)	CV RR-int‡ (%)	SDSD† (ms)
Subclinical hyperthyroidism patients (n=25)	23.4 ± 7.3	1.85 ± 0.42	903 ± 135	4.46 ± 1.67	30 ± 13
Hyperthyroid patients (n=15)	27.5 ± 11.1	1.70 ± 0.37	621 ± 102	2.86 ± 0.80	9 ± 3
Healthy controls (n=15)	19.0 ± 4.3	1.40 ± 0.26	936 ± 114	6.66 ± 3.76	45 ± 28
Effect size for normalization of parameter	- 19.3%	- 24.3%	16.7%	43.0%	47.4%
Population required (n per group)	17	7	6	11	22

Data is expressed as mean ± SD. The bottom part of the table indicates the effect size needed for each for complete normalization for patients with subclinical hyperthyroidism and the size of the population that would be required to detect this normalization.

VMA: vanillylmandelic acid; CV RR-int: coefficient of variation of the RR-intervals (measure of overall heart rate variability); SDSD: standard deviation of the differences in subsequent RR-intervals (measure of beat-to-beat heart rate variability).

** ANOVA p=0.03, # ANOVA p=0.003, & ANOVA p<0.001, ‡ ANOVA p=0.0018, † ANOVA p<0.001*

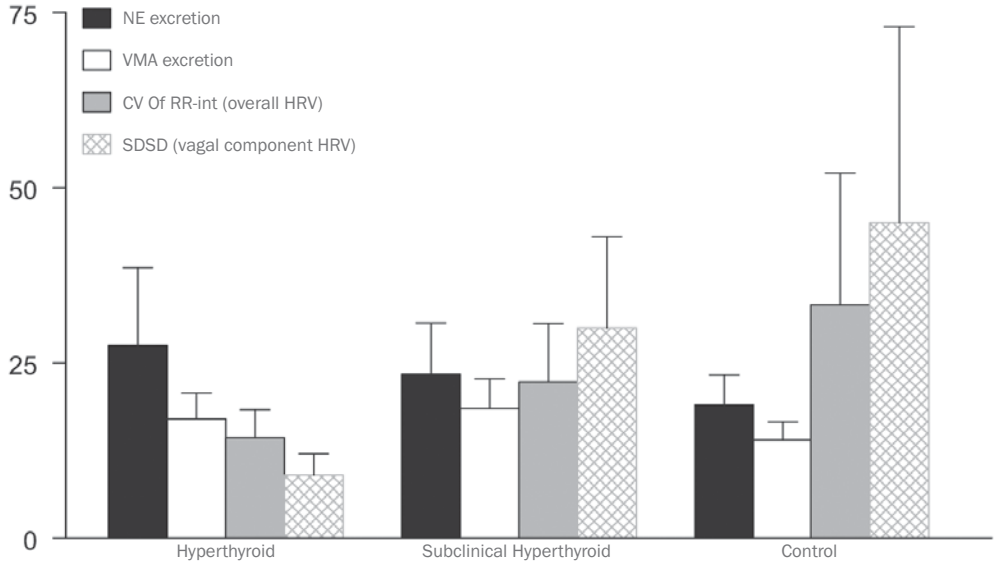
ms (-4.13/+29.98; p=0.142) lower. The findings are summarized in figure 2 which shows that the patients with subclinical hyperthyroidism have intermediate urinary excretion of NE excretion compared to patients with thyrotoxicosis and controls. Also, the measures for overall heart rate variability and the parameter reflecting the vagally mediated component of heart rate variability (SDSD) are between the values of the patients with frank hyperthyroidism and control subjects.

Table 4 also shows the effect size and the size of the population that would have been required if restoration to euthyroidism in the subclinical hyperthyroidism patients would have resulted in complete normalization of the effects parameters.

Discussion

This study was performed to investigate the long-term effects of exogenous subclinical hyperthyroidism on the autonomic nervous system and the potential effects of restoration of euthyroidism. The autonomic nervous system was characterized by assessment of the urinary catecholamine excretion and by heart rate variability parameters. Our study is the first prospective, placebo-controlled randomized study in which the effects of restoration of euthyroidism on the autonomic nervous system in patients with long-term exogenous subclinical hyperthyroidism were studied. The main finding of the study was that restoration to euthyroidism in patients with long-term subclinical hyperthyroidism due to TSH suppression had no appreciable influence on the autonomic nervous system. Urinary catecholamine excretion, the heart rate variability parameters in the time domain and the response to a mental stress test remained virtually unchanged between the patients who remained on TSH suppression and those in whom biochemical euthyroidism was restored.

Figure 2. Graph (mean ± SD) for urinary norepinephrine excretion (in mmol per mmol creatinine), urinary VMA excretion (in mmol per mmol creatinine), overall heart rate variability (CV of RR-intervals in %) and the vagal component of heart rate variability (SDSD in ms) for patients with overt hyperthyroidism, subclinical hyperthyroidism and control subjects. The values for urinary VMA excretion were multiplied 10 times for legibility reasons. The values for CV were multiplied 5 times for legibility reasons.



If the current data are compared with data obtained using similar methodology reported earlier by our group (5), values for activation of the autonomic nervous system in the current patient group with subclinical hyperthyroidism seem to be in between the group of patients with thyrotoxicosis and healthy controls. There were some differences between the groups particularly with regard to age and gender distribution. However, these are not confounding factors. It has been shown that the autonomic nervous system and its activity are not substantially influenced by gender (16). Admittedly, there are reports indicating that increasing age is associated with a decrease in heart rate variability (17,18) due to an age-related decline in parasympathetic regulation (19). These reports however show that this decline occurs over the age range of 20 to 80 years and that the change in heart rate variability occurring in the age range of the population that we studies is very small (17).

We could show that urinary NE excretion in the patients with subclinical hyperthyroidism was indeed lower compared to patients with overt hyperthyroidism and higher compared to the healthy controls. This seems at odds with data reported by Mercuro *et al*. (20) who showed that plasma NE concentrations were significantly lower in patients with exogenous subclinical hyperthyroidism than in controls. However, these data are based on plasma NE concentrations in a single sample of venous forearm blood, while it is known that catecholamine levels are more appropriately determined in arterial(ized) blood, inasmuch as extraction from venous circulation occurs across various organs (21,22), while the urinary excretion of catecholamines and their metabolites is considered to better reflect their average plasma concentrations and whole body turnover in plasma (22,23). We also showed that the heart rate, its total variability (coefficient of variation) and the vagally mediated influence on heart rate variability (SDSD) of the patients were between the values found for patients with thyrotoxicosis and healthy controls.

Interestingly, however, restoration to the euthyroid state in subclinical hyperthyroidism patients did not result in relevant changes in most autonomic nervous system parameters. Apparently, restoring a biochemical euthyroid state in patients who have been subclinical hyperthyroid for >10 years is not reflected in a state of the autonomic nervous system state that is identical to the situation in healthy euthyroid subjects.

It is important to note that in the present study, the intervention of restoring euthyroidism in the patients was successful. TSH concentrations normalized and FT3/FT4 concentrations decreased by approximately 40% and 30% respectively and became in the normal ranges utilized by the laboratories of our hospital. Obviously, it is of crucial importance that the study was sufficiently powered to detect relevant differences. As it was impossible to perform *a priori* power analysis because of lack of data, a population size was chosen which at least would allow exploratory analyses. Subsequently the data that were obtained were used to perform a post-hoc power analysis. This analysis showed that the present study was sufficiently powered to detect differences that would have changed most of the parameters in the Subclinical hyperthyroidism patients to the normal values for these parameters. In order to demonstrate normalization of the urinary NE and VMA excretion two groups of 17 or 7 patients respectively would have been needed. Also for the heart rate variability parameters, it seems that the study was sufficiently powered as for normalization of the RR-interval two groups of 7 patients were required and for the normalization of the overall heart variability (CV) two groups of 6 patients would have sufficed. Admittedly, more patients, (namely 22 patients per group) would have been necessary to also demonstrate normalization of the parameter that is commonly used to characterize the beat-to-beat variability. Nevertheless, we would like to argue that the current study was sufficiently powered for most of the parameters. We feel that our approach in which the restoration to euthyroidism in a homogenous group of patients with exogenous subclinical hyperthyroidism was studied, in a seemingly sufficiently powered randomized experiment is the most appropriate approach to study the effects of subclinical hyperthyroidism on the autonomic nervous system.

Notwithstanding this, the interpretation of these findings is not straightforward. It could be that irreversible changes or adaptation occurs during long term exposure to excess thyroid hormone. If the latter would be true, this would imply that restoration of the autonomic nervous system set-point takes a longer time than half a year. This may explain the difference with studies with a shorter duration in overt hyperthyroidism (5,6) or subclinical hyperthyroidism (13). In addition, another probably crucial difference between our and other studies is that our study the population of patients with subclinical hyperthyroidism was homogenous regarding etiology and duration of the syndrome whereas in other studies more heterogeneous patient populations or populations with endogenous subclinical hyperthyroidism are studied.

In conclusion, long-term exogenous subclinical hyperthyroidism affects the autonomic nerve system as measured by heart rate variability and urinary catecholamine excretion. No differences were observed 6 months after restoration of euthyroidism. This may indicate irreversible changes or adaptation during long-term exposure to excess thyroid hormone that are not remedied by 6 months of euthyroidism. To explore this further additional research is needed.

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