Research paper

Autonomic nervous system and cardiovascular risk assessment during one year of growth hormone replacement therapy in adults with growth hormone deficiency

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ABSTRACT

OBJECTIVE: This prospective study aimed to evaluate the impact of growth hormone deficiency (GHD) on cardiac autonomic tone and on cardiovascular risk and the changes after 12 months of GH replacement therapy (GHRT). GHD is associated with increased cardiovascular morbidity and mortality. This has been attributed to increased markers of cardiovascular risk and to abnormalities of both the cardiac and peripheral autonomic nervous systems. The autonomic cardiac nervous system (ACNS) can be indirectly evaluated by analysis of heart rate variability (HRV) in clinostatism and orthostatism. **DESIGN:** We compared 14 GHD patients at baseline and after 12 months of GHRT and 17 healthy controls. We analyzed a number of cardiovascular risk factors and we used analysis of HRV during the Tilt Test that identified high frequency (HF) and low frequency (LF), representing parasympathetic and sympathetic activity, respectively. **RESULTS:** Compared with the control group, in either clinostatism or in orthostatism our patients showed a significantly lower value of LF ($P=0.047$; $P=0.004$, respectively), whereas HF was significantly reduced in orthostatism ($P=0.037$), and indicatively in clinostatism ($P=0.065$). These values remained unchanged after 12 months of GHRT. No statistically significant differences were found between the LF/HF ratio in untreated and treated patients. In GHD patients, there was a significant reduction of cardiovascular risk in 12 months of replacement therapy ($P=0.002$). **CONCLUSIONS:** Our study highlights the absence of sympathovagal imbalance in GHD patients; GHRT does not change ACNS during the first year of GH treatment but it reduces the absolute cardiovascular risk in these patients.

Key words: Autonomic nervous system, GHD, IGF-I

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Received 05-03-2014, Accepted 05-06-2014

INTRODUCTION

It is known that patients with hypopituitarism have an increased risk of morbidity and mortality from cardiovascular and cerebrovascular disease.¹⁻³

This elevated risk has been attributed to increased cardiovascular risk factors,⁴ such as abnormal body composition,⁵ dyslipidemia,⁶⁻⁸ hypertension, increased premature atherosclerosis and cardiac dysfunction.^{1,9-12} However, two studies have reported that abnormalities both in the cardiac autonomic nervous system¹³ and in peripheral sympathetic activity may contribute to the increased risk of cardiovascular morbidity in growth hormone deficiency (GHD) patients.¹⁴ Indeed, in hypopituitarism, sympathovagal imbalance is generally due to an inadequate replacement therapy with glucocorticoid, thyroid hormones and gonadal steroids, whereas the involvement of growth hormone (GH) is still controversial.¹⁵

The cardiovascular function in GHD can be negatively influenced directly by the decrease of diastolic filling, cardiac performance and contractility, and indirectly by the induced hypercoagulability, insulin resistance, truncal obesity, decreased muscle performance, dyslipidemia, reduced pulmonary capacity and early features of atherosclerosis.¹⁶ Indeed, increase of intima-media thickness of the carotid arteries and decreased physical capacity have been shown in these patients.¹⁷ Conversely, the positive effects of GH replacement therapy are well known and several studies have documented that GH administration may have a trophic effect on health.¹⁸⁻²⁰

A significant relationship has been reported in many pathophysiological conditions between autonomic nervous system imbalance and cardiovascular mortality, including sudden cardiac death.²¹⁻²³ Some studies have shown that cardiac autonomic tone could be altered in patients with myocardial infarction^{24,25} and heart failure²⁶ undergoing percutaneous transluminal coronary angioplasty.²⁷ Sympathovagal imbalance is also a common finding in diabetes and the usefulness of standard cardiovascular tests in assessment of cardiac autonomic neuropathy in diabetes is well documented.²⁸

There is evidence implicating autonomic system dysfunction in cardiovascular disease. Indeed, cardiovascular dysfunction has been linked to diabetic autonomic neuropathy in diabetic patients.²⁹ It has been suggested that early recognition of autonomic dysfunction is important in cardiovascular risk assessment and management.³⁰

Experimental data are available on the impact of GH excess on heart rate variability in transgenic mice overexpressing bovine GH. These animals, representing an experimental model of acromegaly, GH suppresses sympathetic tone, most probably by reducing the availability of norepinephrine.³¹ A recent study by our group has demonstrated that acromegalic patients present with sympathovagal imbalance (due to vagal hypertone) similar to that found in diabetics.³² There are indications that the somatotrophic axis is involved in the regulation of sympathetic activity.³³⁻³⁵

One common and easy technique for the study of the autonomic nervous system is the evaluation of heart rate variability (HRV) during postural changes. Clinical and experimental data suggest that this technique is a powerful predictor of future arrhythmic events.³⁶ There is one important study showing an impairment of sympathetic tone in adult patients with GHD that normalized after 6-12 months of GHRT; however, in this work the time of GHD onset in the enrolled patients was mostly longer than 2 years (median about 13 years).³⁷

The aim of the present study was to prospectively evaluate the heart autonomic tone in patients with recent onset of GHD and the effect of a 12 month GH replacement therapy on it.

SUBJECTS AND METHODS

Patients and protocol

The study group comprised 14 patients with a diagnosis of GHD (7 women, 7 men; age range 28-81 years) selected from a cohort of 150 hypopituitary patients. Highly restrictive inclusion/exclusion criteria were used in order to enroll only cases without additional cardiovascular risk factors.

Inclusion criteria were: diagnosis of adult GHD established by GH response to the insulin tolerance test (ITT) <3 ng/ml or by an inadequate peak after GHRH/arginine stimulation (Guidelines of the Growth Hormone Research Society, 2007); subject older than 18 years old. Any other deficient pituitary axes were adequately replaced in all patients before study entry.

Exclusion criteria were: GHD onset more than

2 years previously, prior rGH therapy, obesity, diabetes mellitus, myocardial infarction, heart failure, hypertension, smoking or any medication that might interfere with autonomic regulation, family history of cardiovascular disease. The GHD patients were compared to 17 healthy controls (8 women, 9 men; age range 26–75 years) selected using the same criteria.

Apart of the assessment of heart autonomic tone, body mass index (BMI), systolic and diastolic blood pressure, IGF-I, lipid profile, blood glucose and glycated haemoglobin (Hb1Ac) were also measured at baseline and after 12 months of rGH treatment (average initial dose of 1.2 ± 0.19 mg/week) titrated on the basis of IGF-I value at 3–6–12 months. Our ethics committee had approved the protocol and written informed consent was obtained from the patients.

Autonomic tests

Autonomic tests were performed by power spectral analysis of heart rate variability in clinostatism and orthostatism, using a frequency domain method. HRV, which is the variability in R-R interval from one heartbeat to the next, is a measure of autonomic nervous system balance.³⁸

An electrocardiographic recording of R-R intervals was made at 10:00 h in a quiet room reserved for this purpose, at least 1 h after venipuncture for routine hormonal evaluation. Subjects were placed supine on a mechanically driven tilt-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, clinostatic R-R intervals were obtained (clinostatism values). Immediately thereafter, each patient was slowly and passively brought into the upright position by rising the table over a 30-sec period and the recording was repeated (orthostatism values). In both clinostatic and orthostatic postures, the EKG was recorded for 330 sec by means of an electrocardiograph connected to a PC equipped with software that sampled the analogical signal at about 200 Hz using an analogic/digital converter. Each R-R interval was measured in milliseconds and memorized as a tachogram (R-R interval duration vs. number of heartbeats). Two series of data, corresponding to clinostatic (in tachogram A) and orthostatic (in tachogram B), R-R intervals, were analyzed in all subjects using a parametric method

based on the autoregressive model for the quantification of HRV signals. The main power densities in the high frequency (HF; 0.15–0.4 Hz) and low frequency (LF; 0.04–0.15 Hz) bands were identified for each density spectrum. In addition, LF/HF ratios were calculated in both clinostatism and orthostatism. An LF/HF greater than 1 is considered normal.

Power spectral analysis identifies 3 peaks of power: a peak of HF, which expresses vagal activity; a peak of LF, which expresses sympathetic activity; and a peak of very LF, which is of uncertain significance. LF and HF are quantitative indicators of neural control of the sinoatrial node; in particular, the LF component is a marker of sympathetic modulation, whereas the HF component is a marker of vagal modulation. LF/HF is regarded as an index of sympathovagal balance in the frequency domain.^{24,25,30,39} All HRV parameters were measured in square milliseconds.

Coronary risk

Absolute cardiovascular risk assessment provides an estimate of the probability, expressed as a percentage, of developing a first major cardiovascular event over the next 10 years based on a number of risk factors. Cardiovascular risk charts have been elaborated from these mathematical functions, considering major cardiovascular risk factors: sex, age, blood pressure, total and HDL cholesterol levels and cigarette smoking.⁴⁰ This chart (Risk Card 2009) offers the option of evaluating both protective factors (as for instance HDL cholesterol levels) and risk factors in the cardiovascular risk assessment.⁴¹

Statistical analysis

Statistical analysis was performed using the SPSS 11.0 program. All data are presented as mean $\pm$ SD. The observed values for each variable in patients and controls group were analyzed using the Mann-Whitney test for independent data. The observed values for each variable in patients before and after treatment were analyzed using the Wilcoxon test for paired data. The correlations between variables were assessed using the Pearson correlation coefficient. A p value < 0.05 was considered statistically significant.

RESULTS

As expected, IGF-I levels before treatment were

significantly lower than normal and increased significantly after 1 year of GHRT (IGF1SDS -1.28 ± 0.40 ; absolute values 82.61 ± 12.13 ng/ml vs. IGF1SDS 0.39 ± 1.07 ; absolute values 156.76 ± 16.04 ng/ml, respectively. P IGF1SDS = 0.002; P IGF1 absolute values = 0.002).

Autonomic tests

HRV values in patients, before and after 1 year of therapy, and controls are shown in Table 1. HF (vagal activity) decreased in all subjects during the test. Compared with the control group, in clinostatism the patients showed a significantly lower value of LF ($P=0.047$) and a reduced, though non-significantly, HF value ($P=0.065$); in orthostatism the values of LF and HF were significantly reduced in GHD patients compared to the normal subjects ($P=0.004$ and $P=0.037$, respectively) (Figure 1). No statistically significant differences were found between the LF/HF ratio in untreated patients and controls, both in clinostatism and orthostatism (Figure 2). LF, unlike what was found in controls, was reduced in the transition from clinostatism to orthostatism, although not significantly (Figure 1). No significant changes were observed after 12 months of GH replacement therapy (Figures 1, 2).

Cardiovascular risk factors

BMI was between 25 and 30 kg/m² at baseline and after 1 year of treatment. Total cholesterol, and triglycerides levels were slightly above normal ranges (200 mg/dl and 150 mg/dl, respectively); HDL cholesterol was normal (>40 -50 mg/dl in males and females,

respectively) and did not significantly change after therapy ($P=0.662$; $P=0.696$; $P=0.181$, respectively). However, a downward trend in total cholesterol and upward trend in HDL was evident. Conversely, LDL cholesterol was significantly reduced ($P=0.022$) (Table 2).

Systolic pressure was normal (<140 mmHg) but higher before therapy start, while it was significantly decreased after one year of treatment (136.43 ± 3.41 mmHg vs. 126.8 ± 3.87 mmHg, respectively, $P=0.005$). In addition, the diastolic pressure showed a significant decrease after treatment (82.50 ± 2.76 mmHg vs. 78.21 ± 1.45 mmHg, respectively, $P=0.003$) and reached values comparable to those found in the control group (76.71 ± 1.46 mmHg).

Blood glucose levels did not significantly increase ($P=0.421$), whereas Hb1Ac values were significantly increased ($P=0.023$), but remained within the normal range ($<6\%$).

All patients were treated with levothyroxine (dose range: 50-150 mcg daily) and cortisone acetate (dose range: 25-75 mg daily) and displayed adequate levels of fT4, sodium - potassium, and urinary 24 hours free cortisol (Table 3). Three of seven female patients were taking replacement estrogen-progestinic therapy given in the premenopausal period (one transdermal, two oral route). All male patients were taking substitutive testosterone therapy (four transcutaneous and three by intramuscular route). During the 12 months of the study, no changes in the doses of these therapies were necessary since the hormonal assessment constantly

Table 1. HRV parameters in patients and controls. LF and HF values are expressed as mean standard deviation

		Patients baseline	Patients after 12 months GHRT	Controls
	AGE RANGE (years)	28-81	29-82	26-75
	IGF-1 SDS	-1.28 ± 0.40	0.39 ± 1.07	
Clinostatism	LF (ms(2)/Hz)	678 ± 543	572 ± 575	1138 ± 667
	HF (ms(2)/Hz)	317 ± 294	254 ± 183	711 ± 602
	LF/HF	2.82 ± 1.73	2.66 ± 1.95	2.94 ± 2.86
Orthostatism	LF (ms(2)/Hz)	508 ± 539	431 ± 278	1251 ± 456
	HF (ms(2)/Hz)	200 ± 157	142 ± 116	416 ± 274
	LF/HF	2.62 ± 1.72	3.60 ± 2.50	4.78 ± 3.82

LF: low frequency; HF: high frequency; GHRT: GH replacement therapy.

Figure 1. Box and whisker plots of LF and HF in both clinostatism (a) and orthostatism (b) in the study groups. The *lines in the boxes* represent the median; the *boxes* are the 25-75th percentile range and the *whiskers* are the 10-90th percentile range. LF: low frequency; HF: high frequency.

Figure 2. Box and whisker plots of LF/HF in both clinostatism and orthostatism in the study groups. The *lines in the boxes* represent the median; the *boxes* are the 25-75th percentile range and the *whiskers* are the 10-90th percentile range. LF: low frequency; HF: high frequency.

Table 2. Variations in levels of cholesterol (Total, LDL, HDL), triglycerides, BMI, systolic and diastolic blood pressure before and after 12 months of GH replacement therapy expressed as mean SEM

	Baseline	12 months	P
Chol TOT (mg/dl)	223,2±8,9	214,3±7,2	0.662
Chol LDL (mg/dl)	142,8±7,4	125,0±5,5	0.022
Chol HDL (mg/dl)	47,4±2,8	54,0±3,6	0.181
TG (mg/dl)	164,2±20,2	176,5±26,3	0.696
BMI (kg/m ²)	27,64±1,03	27,40±1,00	0.836
SP (mmHg)	136.43±3.41	126.8±3.87	0.005
DP (mmHg)	82.50±2.76	78.21±1.45	0.003

Chol tot: total cholesterol; Chol LDL: LDL cholesterol; Chol HDL: HDL cholesterol; TG: triglycerides; BMI: body mass index; SP: systolic blood pressure; DP: diastolic blood pressure.

Table 3. Variations in levels of urinary cortisol, plasmatic sodium and potassium, plasmatic testosterone and ft4 before and after 12 months of GH replacement therapy expressed as mean± standard deviation

	Patients (baseline) mean±SD	Patients after (12 months of therapy) mean±SD
Number of subjects	14 (7M; 7 F)	14 (7M; 7 F)
AGE	55.24±17.56	56.24±17.56
Free urinary cortisol/24h (mcg/24h)	90.11±22.05	82.98±29.56
Na (mEq/L)	139.82±1.4	140.27±1.2
K (mEq/L)	3.8±0.5	4.0±0.4
Testosterone (ng/dl) (only males)	389.98±132.01	327.55±187.12
ft4 (ng/L)	11.26±3.7	12.65±2.9

Na: sodium; K: potassium; ft4: free thyroxin; M: male; F: female; SD: standard deviation.

displayed an adequate replacement of all the axes. Although the 10-year absolute coronary risk was not elevated in any of the patients (8.8±7.4%) at diagnosis, the value of risk after one year of therapy was reduced in 12 of the 14 patients with GHD (6.6±4.6%), and particularly in those displaying the higher values before the start of treatment with rGH. Therefore, these data show a significant reduction of cardiovascular risk in 12 months of replacement therapy (P=0.002).

DISCUSSION

GHD can lead to cardiovascular events and thus to increased mortality.¹⁻³ Early diagnosis, treatment and follow-up enable the reduction of this risk.¹⁸⁻²⁰ As in this study we have considered only non-diabetic, non-smoking patients without any known heart disease or history of cardiovascular events, the role of GH per se was highlighted without confounding factors. In our study, we prospectively evaluated the autonomic system, lipid and metabolic profile and blood pressure to explore the “pure” role of GHD status on cardiovascular risk in an early phase of disease. In fact, the effect on cardiac function in patients treated with GH is unknown so far. The data available in the literature primarily refer to long-term treated patients who already display impaired cardiovascular function. Surprisingly, the rather precocious lipid profile and blood pressure modifications in these patients suggest that even a short-term period of GHD in patients without clinically evident vascular alterations results in an increased risk of coronary disease. Moreover, the lack of correlation between the GHRT and autonomic function suggests that increased cardiovascular mortality, reported in patients with GHD, does not seem dependent on dysregulation of the autonomic system, this being at variance with acromegalic and diabetic patients.^{29,31,32} Nevertheless, it must be underlined that our data describe no clinically significant alterations in sympathovagal balance in an early phase of GHD onset, while other studies have demonstrated it in longer disease duration.³⁷ This could be explained by the fact that GHD disease duration is the most important risk factor; indeed, the differences (HF and LF values significantly reduced) observed in our patients could predict, after more years of untreated GHD, significant alterations also from the clinical point of view (like a sympathovagal imbalance if reduction in LF became more substantial than that in HF). An important study analyzing the role of GHD in the autonomic nervous system demonstrated a reduced beta adrenoreceptor responsiveness in GHD rats despite a normal myocardial beta-adrenoreceptor density.⁴² The latter, together with impairment of left ventricular performance, which is known to be present in GHD patients, may identify alterations in the autonomic nervous system if GHD is not adequately treated.

Moreover, 12 months' treatment with rGH significantly reduced the risk of a coronary event in 10 years due to improvement of modifiable risk factors (blood pressure and lipid profile), even at an early stage of GHD disease. Consistently, an improvement in blood pressure and lipidic profile after GHRT was demonstrated in other studies.^{16,43}

The risk reduction induced by therapy, even in patients slightly compromised, stresses the importance of starting GH replacement at early stages also in patients apparently free from risk factors. One limitation of the study is the small number of patients, justified by the rarity of the disease and the strict criteria of recruitment. However, our series is perfectly adequate for the purpose of the study and devoid of confounding factors as well.

In conclusion, our data show that, in a meticulously selected group of patients, one year of rGH therapy improves cardiovascular risk by preventing the onset of atherosclerotic processes in patients with recent-onset GHD. The study also demonstrates that patients with recent GHD do not exhibit sympathovagal imbalance. However, we cannot exclude that GHD status untreated for more than two years could affect the autonomic nervous system, as previous studies have reported. Larger and longer-term case studies may help to explain the pathophysiological mechanisms that regulate the influence of GH and IGF-I on the autonomic system, particularly in patients with long-standing GHD. Indeed, it is likely that the discrepancies described in the literature by other authors are due to longer periods of GHD in their series.

ACKNOWLEDGEMENT

This paper is dedicated to Prof. Francesco Minuto, our beloved "maestro" who passed away a few months ago.

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