ALTERED AUTONOMIC CARDIAC REGULATION IN INDIVIDUALS WITH DOWN SYNDROME

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Running head: Autonomic cardiac control and Down syndrome

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ABSTRACT

This study was designed to test the hypothesis that individuals with Down syndrome, but without congenital heart disease, feature an altered autonomic cardiac regulation.

Ten subjects with Down syndrome (DS) and ten gender-and age-matched healthy subjects (NR) were studied at rest and during active orthostatism, which is known to induce reciprocal changes in sympathetic and parasympathetic traffic to the heart. Autoregressive power spectral analysis was used to investigate RR-interval variability. Baroreflex modulation of sinus node was assessed by the spontaneous baroreflex sequences method.

No significant differences were observed in arterial blood pressure both at rest and in response to standing between DS and NR. Also RR-interval did not differ at rest. RR-interval decreased significantly less during standing in DS in comparison to NR. Normalized low-frequency (LF_{NU}) and high-frequency (HF_{NU}) component of RR-interval variability did not differ at rest between DS and NR. During standing, a significant increase in LF_{NU} and decrease in HF_{NU} was observed in NR, whereas no significant change in both LF and HF components of RR-interval variability was detected in DS. At rest, baroreflex sensitivity (BRS) did not differ between DS and NR, and underwent a significant decrease on going from supine to upright in both group. However, BRS was greater in DS than in NR during standing.

These data indicate that subjects with DS exhibit reduced HR response to orthostatic stress associated with blunted sympathetic activation and vagal withdrawal and with a lesser reduction in BRS in response to active orthostatism. These findings suggest an overall impairment in autonomic cardiac regulation in DS and may help to explain the chronotropic incompetence typically reported during exercise in subjects with DS without congenital heart disease.

Key words: autonomic nervous system; arterial baroreflex; heart rate variability; orthostatic stress
INTRODUCTION

It has recently been suggested the subjects with Down syndrome (DS) not suffering from concomitant congenital heart disease may feature a dysfunction in autonomic cardiac regulation, which would manifest mainly with a reduced heart rate (HR) response to excitatory stimuli (4). Specifically, studies dealing with work capacity in subjects with DS, reported a reduced HR response to exercise (5-7,13,14), which has been tentatively explained by a blunted sympathetic activation. Recently, Fernhall and Otterstetter (4) reported reduced blood pressure (BP) and HR responses to cold pressor test and static handgrip in adults with DS without congenital heart defects. On the basis of these hemodynamic responses, the authors speculated that subjects with DS have a reduced sympathetic activation associated with a reduced vagal withdrawal. In contrast, Udeschini et al (39) observed no significant differences in BP and HR responses to cold pressor test between subjects with DS and controls. Similarly, whereas one recent study (8) observed a reduced HR response to head-up tilt, other studies have shown no differences in response to active orthostatism between individuals with DS and healthy controls (39). Hence, although there is some evidence of an impaired autonomic cardiac regulation in response to excitatory stimuli in subjects with DS, the data are still conflicting. In addition, the more subtle mechanisms of this impairment have received less attention. In fact, studies performed so far, made inferences based on the target organ response, that is HR. Only few studies directly assessed sympathetic and vagal cardiac modulation in awake subjects with DS, reporting no differences in autonomic cardiac modulation at rest and a reduced vagal withdrawal in response to brief isometric handgrip (10) and submaximal dynamic exercise (1), without differences in cardiac sympathetic activation in these patients in comparison to healthy individuals (10) or people with mental retardation without DS (1). Finally, there is no information on the baroreflex control of the sinoatrial node, that is a key component of cardiovascular homeostasis to challenging stimuli and carries relevant pathophysiological and prognostic information (23,29). A more thorough understanding of autonomic cardiac regulation in subjects with DS might have important clinical implications, because life expectancy is greatly enhanced in this patients population (3), and alterations in autonomic cardiac regulation are associated with an increased cardiovascular risk (16).
Therefore, in this study we investigated the HR response to active standing, a stimulus known to induce reciprocal changes in sympathetic and vagal outflow, in subjects with DS to test the hypothesis that all the mechanisms of neural cardiac regulation are impaired in this patients population.

**METHODS**

*Subjects.* We studied 10 subjects with Down syndrome (6 females and 4 males), mean age 26.3 ± 2.3 years, body mass index 26.2 ± 1.1 and 10 healthy volunteers (6 females and 4 males) mean age 26.1 ± 4.0, body mass index 23.9 ± 0.3 ($P = 0.064$ vs DS). Subjects with Down syndrome were recruited from The Developmental Disability program of Pediatric Neurorehabilitation Division of the I.R.C.C.S. San Raffaele, where they had been screened for cardiovascular diseases through physical examination, resting electrocardiogram and echocardiographic examination. Patients were also free from any diseases that could influence the autonomic nervous system, e.g. diabetes or other metabolic diseases, asthma or other respiratory disorders. No one subject had severe mental retardation. No one subject was involved in regular physical activity.

All control subjects were normotensive, taking no medication and were free from any disease based on medical history and physical examination. All subjects were non smokers and not involved in regular physical activity. All participants gave their informed consent to the study, after the nature of the study was explained. For subjects with DS, their parents signed informed consent. The protocol was approved by the Ethics Committee of the I.R.C.C.S. San Raffaele Pisana.

*Recorded variables.* The electrocardiographic signal was recorded from a precordial chest lead. Arterial blood pressure was continuously and non-invasively measured from the third finger of the hand using the plethysmographic method of the unloaded arterial wall (Finapres, Ohmeda 2300 NIBP monitor, USA). Respiratory signal was recorded by means of a thoracic belt (Biopac). The three analogue signals were sampled at 300 Hz per channel (Biopac systems) and stored on the hard disk for subsequent analyses.
Protocol. The experiments were performed in the morning in a laboratory at ambient temperature (22-24 °C). The subjects were required not to eat or to drink coffee for at least two hours. After instrumentation, the subjects lay supine for 15 min before experiments to relax in the room made dark and noiseless. The experiments consisted of 10 min of supine rest followed by 10 min of active orthostatism (STAND). With the subject supine the arm with the instrumented finger was held extended in the mild-axillary position. The elbow was also held by a padded support, while the forearm was free. In the standing posture the arm with instrumented finger was held extended by an adaptable support with the hand at heart level, taken as the fourth intercostal space (19).

Power spectral analysis. The methodology for spectral analysis has been described in detail previously (17). Briefly, a derivative-threshold algorithm provided the continuous series of RR-interval (tachogram) from the ECG signal. The harmonic components of RR-interval variability were evaluated by the autoregressive method (model order 8-12). Components in the frequency band from 0.03-0.15 Hz were considered as low-frequency and those in the range 0.15-0.4 Hz, which are synchronous with respiration, as high-frequency. Oscillations slower than 0.03 Hz were considered as very-low frequency (VLF) components. The power density of each spectral component was calculated both in absolute values and normalized units (31, 38), the latters obtained by dividing the absolute power of each spectral component by total power, after having subtracted from it the power of the DC component, if present, and multiplying this value by 100. The normalization procedure is particularly helpful in allowing comparisons between subjects or experimental conditions characterized by differences in total power or in DC noise (31). LF and HF component of R-R interval variability, in normalized units, are considered an expression of sympathetic and vagal modulation to the sinoatrial node, respectively (18, 26, 31, 32, 38). Spectral analysis of the respiratory signal was performed on the signal sampled once for every cardiac cycle, employing a procedure similar to that described for RR-interval. Respiratory spectra were used to assess the main respiratory frequency and to locate the respiratory component of the power spectral analysis of R-R interval variability.
Spontaneous baroreflex analysis. Details of this analysis have been previously described (17-19). Briefly, the beat-by-beat time series of systolic blood pressure (SBP) and RR-interval were scanned by a computer to identify sequences of three or more consecutive beats in which SBP and RR-interval change in the same direction, either increasing (+RR/+SBP) or decreasing (-RR/-SBP). A linear regression was applied to each individual sequence and only regressions with linear $r^2$ values $> 0.85$ were accepted. The mean individual slope of the SBP/RR-interval relationship, obtained by averaging all slopes computed within a given test period, is then calculated and taken as a measure of the integrated baroreceptor reflex sensitivity for that period (2,33). This method has provided reproducible results during many laboratory tests, including active orthostatism (19).

Statistics. Each variable was checked for normality of distribution by the Kolmogorov-Smirnov test. When normality test passed, parametric statistical analyses, that included Student’s paired and unpaired $t$-tests were used. When the test failed, non-parametric statistical analyses, that included Wilcoxon test and Mann-Whitney rank sum test were used. Since the major part of autonomic variables had distributions that were significantly different from normal, non-parametric statistical tests were used for these variables. Values are presented as means ± SEM and as median and interquartile range, as appropriate. Differences were considered statistically significant when $P < 0.05$. 

RESULTS

Cardiovascular responses to standing (Table 1). Arterial pressure (AP) was not significantly different in the supine posture between DS and controls. During standing, AP did not change significantly from supine in both groups. Supine RR-interval was not significantly different between DS and controls. In both groups, RR-interval decreased significantly during standing; however, RR-interval was significantly less in controls than in subjects with DS, and the magnitude of the RR-interval decrease was significantly greater in controls than in subjects with DS (199.3 ± 82 vs 130.5 ± 56 msec, $P = 0.04$).

Power spectral and spontaneous baroreflex analyses (Table 2). RR-interval variance did not differ significantly between DS and controls at rest and underwent a significant decrease during standing in both groups. The tendency towards a greater variance in DS was due to two subjects showing a very large variance. At rest, the HF and LF components of RR-interval variability were not significantly different between the two groups. During standing, control subjects showed significant increases and decreases in the low- and high-frequency RR-interval oscillations (in normalized units) respectively, whereas in subjects with DS no significant changes in both low- and high-frequency oscillations (in normalized units) were detected.

Baroreflex sensitivity (BRS) did not differ significantly between DS and controls in the supine posture and underwent a significant decrease while standing in both groups; however BRS was significantly less in controls than in DS during standing. No significant difference was detected in BRS between increasing (+RR/+SBP) and decreasing (-RR/-SBP) blood pressure ramps either during supine or standing in both experimental groups.

Respiratory rate was not significantly different between DS and control subjects both in the supine posture (0.27 ± 0.02 vs 0.28 ± 0.02 Hz) and during standing (0.29 ± 0.03 vs 0.29 ± 0.02 Hz), with no significant changes on going from supine to standing in both groups.
DISCUSSION

The novel finding of the present study is that neural cardiac regulation in response to a physiological excitatory stimulus is impaired in patients with DS without overt cardiovascular diseases, and involves alterations in both branches of the autonomic nervous system and baroreflex cardiac modulation.

During supine rest, no significant differences were observed in AP, RR-interval and neural cardiac modulation between patients with DS and controls. As expected (19, 20, 28, 31), in healthy subjects, active orthostatism, with the attendant sympathetic activation and decrease in vagal activity, induced a significant decrease in RR-interval, associated with a decrease in the HF and an increase in the LF components (in normalized units) of RR-interval variability, respectively, and a concomitant decrease in BRS. On the contrary, in patients with DS no significant changes in both LF and HF components of RR-interval variability were observed in response to standing and the decrease in RR-interval was significantly less than in controls; in addition, BRS, although reduced when compared to the supine posture, underwent a lesser decrease in patients with DS in comparison to healthy subjects.

These findings clearly point towards an impaired autonomic cardiac regulation in patients with DS, which would not be evident at rest but would be manifested in response to a task like standing affecting the overall autonomic nervous system. The observed results would indicate both a blunted sympathetic activation and a reduced vagal withdrawal in response to orthostatic stress in patients with DS, both of which would contribute to the reduced tachycardic response to standing. The relatively lesser reduction in baroreflex opposition to HR changes at the current, similar, AP levels, as suggested by the significantly greater BRS values during standing in DS in comparison to control subjects, might have also contributed to the reduced HR response.

To our knowledge, this is the first study to provide direct experimental evidence of an impairment of all the mechanisms regulating HR in patients with DS. This finding could provide a clue to explain, in part, the lower HR response to exercise and the low physical work capacity typically
observed in patients with DS (4-7, 10, 13, 14), since HR increase during exercise is due to both a vagal withdrawal and sympathetic activation with a relevant contribution provided by a lessened baroreflex opposition to HR increase, i.e. a decreased BRS (17, 21). This study confirms recent data by Fernhall et al (8) of a blunted HR response to upright tilt in DS, but also extend them in that it provides direct experimental evidence as to the neural mechanisms involved in the blunted HR response to orthostatic stress. In this context, it should be outlined that steady-state HR response to upright tilt and active standing do not differ (40). The present investigation also confirms previous findings by Figueroa et al (10) of no significant differences between individuals with DS and healthy subjects in HRV parameters at rest. Apparently at variance with the above findings, Baynard et al (1) reported a significantly greater HF power at rest in patients with DS in comparison to individual with mental retardation without DS. Although that study cannot be directly compared with the present one because of the lack of a nondisabled control group (1), however it is of note that the difference in resting HF power in the study by Baynard et al (1) was not significant when expressed in normalized units.

In contrast with the present study, Udeschini et al (39) reported no significant difference in HR response to standing between DS and healthy individuals. However, their results were obtained in a very small sample size (n=5) and no data were provided as to the autonomic mechanisms regulating the sinus node rate, making difficult a comparison with our results. In addition, the increase in HR on going from supine to standing in their control group was inappropriately low (7 beat/min) for healthy people (40), raising some doubts on subjects selection.

The present investigation, by its design, cannot define the mechanism(s) responsible for the impairment of autonomic cardiac regulation in DS. However, the consistent findings of abnormalities in brainstem auditory evoked potentials (9, 11), the failure of growth and maturation in the brain from an early age, with loss of neurones (41,42), and dendrites (37), and the damage of neurotransmitter system (27,43), would provide the anatomic substrate for the autonomic dysfunction occurring at central, brainstem, site as a result of the genetic disorder.
Our findings carry potential clinical implications. It has been suggested that chronotropic incompetence could help to explain the higher levels of cardiovascular disease among individuals with DS (34), inasmuch as chronotropic incompetence is associated with increased cardiovascular risk (22, 24, 25), even before heart disease develops (24, 35) in nondisable populations, but the mechanism(s) of chronotropic incompetence is still to be clearly elucidated (24,30,36). The alteration in sympathetic, parasympathetic and baroreflex cardiac modulation observed in the present investigation could provide the physiological basis for the chronotropic incompetence of DS patients, and this may be related to increased risk of developing heart disease in subjects with DS who exhibit chronotropic incompetence. However, at present, it is not known whether chronotropic incompetence and impairment in autonomic cardiac regulation, separately or being related one each other, impact on health outcome in people with DS. Individuals with DS are living longer than in the past (3,15), with a median age of survival which is nowaday of over 60 years (12). Given the longer life expectancy in this population, longitudinal studies may be warranted to evaluate the impact of impaired autonomic cardiac regulation on health outcome in DS patients.

Limitations of the study include the small sample size, that decreases the statistical power of the study. However, the consistency of several reports showing autonomic dysfunction in individuals with DS with the findings of the present study, would support our conclusion of an impairment autonomic control of HR in DS and suggests that our findings are not unique only to DS patients of the present investigation. The possibility that caffeine assumption could have influenced our results cannot be totally ruled out. However, not all subjects, either controls or persons with DS, were used to drink coffee as a part of their breakfast and often experiments were conducted in the morning even more than 2 hours after breakfast. In addition, there was no significant difference in BP, HR and any autonomic variable at rest between controls and subjects with DS. Hence, we believe very unlikely that our findings have been substantially influenced by caffeine ingestion.

A final potential limitation of this study includes the indirect method employed to assess changes in autonomic function. However, the issue of the validity of this approach to assess changes in
autonomic regulation was recently addressed by experiments in humans (32). Direct recordings of muscle sympathetic nerve activity were performed during various states of autonomic regulation, as produced by graded infusions of vasodilators and vasoconstrictors. The presence of similar, coherent, oscillations at low- and high-frequencies in nerve activity and RR interval (as well as in blood pressure) variability at various levels of induced pressure changes provided support to the use of LF_{RR} and HF_{RR}, to obtain information on the changing state of sympathetic and vagal modulation of the sinoatrial node, respectively.

In conclusions, we investigated autonomic mechanisms of cardiac regulation in response to standing in patients with Down syndrome and observed blunted sympathetic activation and vagal withdrawal associated with a lesser reduction in baroreflex opposition to HR changes in response to active orthostatism in this patients population. These findings may help to explain the chronotropic incompetence typically reported in subjects with DS without congenital heart disease.
Grants

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REFERENCES


TABLE 1. Cardiovascular responses to active orthostatism.

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<td>REST</td>
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<tr>
<td>RR-interval, msec</td>
<td>1062.6 ± 61.8</td>
<td>932.1 ± 67.5*</td>
<td>925.1 ± 40.7</td>
<td>725.8 ± 24.6*†</td>
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<td>Systolic blood pressure,</td>
<td>116.1 ± 2.7</td>
<td>115.0 ± 5.5</td>
<td>118.7 ± 2.8</td>
<td>118.5 ± 3.8</td>
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<tr>
<td>Diastolic blood pressure,</td>
<td>73.5 ± 2.3</td>
<td>74.5 ± 3.4</td>
<td>71.7 ± 2.0</td>
<td>78.0 ± 3.5</td>
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Values are given as mean ± SEM. * P < 0.01 vs REST, † P < 0.05 vs Down
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<tr>
<td></td>
<td>REST</td>
<td>STAND</td>
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<tr>
<td>Variance, ms²</td>
<td>4056 (2594-7658)</td>
<td>1787 (1117-3044)*</td>
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<tr>
<td>Low frequency, ms²</td>
<td>1200 (650-2616)</td>
<td>498 (261-774)</td>
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<td>n.u.</td>
<td>51.3 (35.5-59.4)</td>
<td>64.3 (48.6-73.8)</td>
</tr>
<tr>
<td>High frequency, ms²</td>
<td>1102 (365-2216)</td>
<td>185 (131-237)*</td>
</tr>
<tr>
<td>n.u.</td>
<td>34.6 (27.4-49.9)</td>
<td>18.7 (11.4-38.0)</td>
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<tr>
<td>BRS, ms/mmHg</td>
<td>26.0 (19.9-33.1)</td>
<td>13.0 (10.2-15.7)*</td>
</tr>
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Values are given as median and interquartile range. n.u., normalized units; BRS, baroreflex sensitivity. * P < 0.01 vs REST. † P < 0.05 vs Down