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Hormonal and autonomic responses to autonomic stimulation are altered in young men with Down syndrome

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Abstract

Background: Down syndrome (DS) population frequently presents autonomic and hormonal dysfunctions that have not been concurrently investigated, and yet could limit adaptations to stress.

Methods: We compared 11 control (CONT) and 11 DS adult subjects during three autonomic stimulation tests. Heart rate, blood pressure and hormonal concentrations were measured continuously during these tests.

Results: DS subjects showed lower systolic blood pressure in the 3 tests ($p<0.05$) and blunted baroreflex sensitivity in 2 tests. Handgrip test induced increased heart rate ($p<0.001$) systolic blood pressure ($p<0.01$) and norepinephrine ($p<0.05$) in CONT only. During cold pressor test, ACTH and norepinephrine concentrations were higher during immersion than at rest ($p<0.01$ and $p<0.001$ respectively) in DS only. During head-up tilt test, ACTH and cortisol concentrations were higher in DS in both positions ($p<0.05$).

Conclusion: These results support the hypothesis of altered hormonal and vascular responses to autonomic provocation tests in DS subjects.

Introduction

With a prevalence of 1 in 700 live births [1] Down syndrome (DS) is the most common chromosomal genetic disorder. This syndrome affects several systems and is associated with a wide spectrum of cognitive and physiological impairments. Specifically, these characteristics include hypotonia, short stature, overweight, endocrine disorders and dysautonomia [2,3]. The autonomic dysfunction in this population could be associated with increased morbidity and mortality [4,5]. It has been shown that subjects with DS have a blunted cardiovascular response to exercise or autonomic stimulation including chronotropic incompetence, reduced heart rate and blood pressure [6-9]. A reduced sympathetic activation and a blunted parasympathetic withdrawal seem to be involved in these cardiovascular responses [7,10]. Hormonal perturbations could also be partially involved in these altered adaptations [8,9,11]. In fact, epinephrine and norepinephrine concentrations are reduced in DS compared to control (CONT) subjects during a stress such as physical exercise [6,12,13]. This suggests a reduced sympathetic response because heart rate response to a stress is regulated by norepinephrine. In addition, some authors have also observed reduced catecholamine's concentrations during sleep in children with DS compared to typically developed children [10]. Nevertheless, if Jansen *et al.* [14] have established a link between hormonal and autonomic functions in autism spectrum population, this relation has not been widely explored in subjects with DS.

To our knowledge, hormonal and autonomic responses to autonomic tests have not yet been concurrently assessed. Therefore, the aim of this study was to compare autonomic and hormonal responses to 3 autonomic nervous system provocation tests between DS and CONT subjects. We hypothesized that subjects with DS had impaired responses compared to CONT.

Methods

Participants' characteristics

Twenty-two young male participants were recruited: 11 subjects without DS nor intellectual disability (CONT: 22±2 years old, from 20 to 25) and 11 subjects with DS (DS: 22±4 years old, from 18 to 29) who presented free and homogeneous trisomy. The participants did not have cardiac insufficiency nor cardiovascular disease; severe disease (*e.g.* diabetes, leukemia, or obstructive sleep apnea); medication that may alter the cardiovascular or autonomic response; and asthma nor other respiratory disorder, verified on a medical visit. All participants and the legal representatives of the participants with DS received information about the study design and provided their written informed consent. This study was approved by the local ethic committee of the hospital (2009-A00376-51/1).

Height and weight were measured on the first medical visit. This visit permitted subjects to familiarize with the different tests. Body composition was assessed by subcutaneous skin fold measurement using a Lange Skinfold Caliper (Cambridge Scientific Industries, Cambridge, Maryland, USA). Body fat percentage was measured on four skinfold sites: triceps, biceps, subscapular and supra-iliac [15]. Body mass index was calculated as weight divided by height squared (kg/m^2). The physical characteristics of the subjects are shown in Table 1.

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Study design

Three standard cardiovascular reflex tests were used to assess cardiovascular autonomic function, following the same order for all participants: (1) Handgrip + ischemia test (HIT); (2) cold pressor test (CPT) and (3) head-up tilt test (HUTT). A 15-minutes resting time was given between each test (Figure 1).

These three tests were performed during the same morning visit, under the same conditions: in a quiet environment with controlled temperature. Hormonal parameters (cortisol, adrenocorticotropic hormone (ACTH), epinephrine and norepinephrine) were collected using a venous catheter at six different times, at rest and after stimulation: $T0_{\text{HIT rest}}$, $T1_{\text{after HIT}}$, $T2_{\text{CPT rest}}$, $T3_{\text{after CPT}}$, $T4_{\text{HUTT rest}}$, $T5_{\text{after HUTT}}$ (Figure 1).

Heart rate (HR) and blood pressure (BP) were measured continuously in resting and stimulation situations.

During the first period of rest, in a seated position, participants performed a maximum voluntary contraction using a handgrip dynamometer (SMFD500TR 1300N, Sensel Measurements, Vincennes France). The handgrip was held in the dominant hand and allowed to determine the maximal voluntary contraction (Table 1).

1) Handgrip + ischemia test

In a seated position, handgrip was maintained at 40% of the maximum voluntary contraction for 2 minutes using a dynamometer, followed by 3 minutes of peripheral ischemia. Ischemia was obtained by laying a tensiometer inflated on the biceps.

2) Cold pressor test

The participant was in a semi-supine position and his right hand was immerged into ice-cold water (6°C) for 5 min. The participant was instructed to breathe normally and to avoid any muscular contraction of his hand.

3) Head-up tilt test

Table 1. Characteristics of participants at rest.

	CONT	DS
Age (years)	22 ± 2	22 ± 4
Height (cm)	176.1 ± 9.1	158.7 ± 5.7^{aa}
Weight (kg)	67.2 ± 7.3	59.3 ± 7.8^{aa}
BMI (kg.m^{-2})	21.7 ± 2.1	23.5 ± 2.7
Fat Mass (%)	15.7 ± 4.4	19.3 ± 3.6
Resting HR (bpm)	59 ± 7	59 ± 8
SBP (mmHg)	122 ± 12	111 ± 13^{aa}
DBP (mmHg)	81 ± 4	75 ± 8^{aa}
MVC (N)	504 ± 113	242 ± 71^{aaa}

CONT: Control; DS: Down syndrome. ^{aa}p<0.01, ^{aaa}p<0.001 significant difference between CONT and DS. The effect size values are shown in italics and in parentheses. Values are means ± SD. BMI: body mass index; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood pressure; MVC: maximal voluntary contraction

Figure 1. Protocol time frame

09:00 – 09:05		09:20 – 09:25		09:40 – 10:00
HIT	REST 15 min	**CPT**	REST 15 min	**HUTT**
T0 – T1		T2 – T3		T4 – T5
2 min 40% MVC+ 3 min ischemia		Cold immersion 5min 4°C		10 min supine 10 min head-up

HIT: handgrip + ischemia test; CPT: cold pressor test; HUTT: Head-up tilt test; T0: blood sample before contraction; T1: blood sample after contraction; T2: blood sample before immersion; T3: blood sample after immersion; T4: blood sample in supine position; T5: blood sample in head-up position

The test was performed on a motorized tilt table allowing passive position changes. Participants were instructed to breathe normally, to be quiet and to avoid any movement. After 10 minutes in the supine position the participant was head-up tilted to a 70° level on the electrical table for 10 min. The duration of the tilting maneuver from and to the supine position was of 30s for each change of position.

Cardiovascular measurements

Heart rate and blood pressure responses were monitored continuously during the 3 tests.

Beat-to-beat heart rate was measured with a Medical electrocardiogram Monitor cardio (Nexfin HD-BMeye, technology Finapres, Amsterdam; Netherlands). Electrocardiogram electrodes were placed on the chest using a six lead configuration and determined successive R-R intervals (electrocardiogram signal: 1000Hz; recording frequency: 1s).

Beat-to-beat blood pressure in the finger arterioles, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a blood pressure cuff (Nexfin HD-BMeye). The cuff was applied to the middle phalanx of the middle finger.

Heart rate variability (HRV) analysis

R-R intervals were analyzed over two periods of the tests: in a resting period and a stimulation period. Data were exported to the Kubios HRV software (Biosignal Analysis, Department of Applied Physics, University of Kuopio, Finland) which allowed the analysis of HRV. Spectral analysis was performed with a Fast Fourier Transform to quantify the spectral density of the low frequency (LF; 0.04 to 0.15 Hz) and the high frequency (HF; 0.15 to 0.40 Hz) power bands. All these components were expressed in normalized units (nu) [16]. The data acquisition and processing strategy was conformed to consensus panel recommendations for the assessment of cardiovascular variability [16]. In the time domain we obtained the mean heart rate (bpm), the mean R-R interval (ms), the square root of the mean of the sum of the squares of differences between adjacent R-R intervals (rMSSD; ms, (estimate of short-term HRV components) and the proportion of interval differences of successive R-R intervals greater than 50 ms (pNN50; %) that reflects parasympathetic activity [16].

Blood pressure variability (BPV) analysis

The sequential series of successive systolic blood pressure were subjected to a discrete Fast Fourier Transform to yield power spectra of the rhythmic oscillations over a frequency range of 0.02–0.50 Hz, with a resolution of 0.01 Hz. Blood pressure rhythmic oscillations were analyzed with the same recommendations as those described for HRV analysis. For each time segment, the power was calculated for the low frequency band ($LF_{BPV:}$ 0.07–0.15 Hz) [17] in addition to mean SBP and DBP values. In the BPV only the LF band was presented because it reflects sympathetic activity related to vascular tone control [18,19]. The simultaneous blood pressure and heart rate variabilities analysis allowed the calculation of the baroreflex sensitivity index (α_{LF}) as: $\alpha_{LF} = \sqrt{(LF_{HRV}/LF_{BPV})}$ [20] in the 0.07-0.15 Hz range [21].

Biological analysis

Hormonal variables were assayed in plasma samples. The blood samples were immediately centrifuged (3000g, 10min) and aliquoted, frozen and stored at −80 °C before analysis. In order to prevent epinephrine and norepinephrine catabolism we used specific tubes containing protease inhibitor (Becton Dickinson France, Le Pont de

Claix, France). These two hormones were tested by high-performance liquid chromatography with electrochemical detection. ACTH was measured using an IRMA method (Brahms kit with a sensitivity of 0.5pmol/L).

Statistical analysis

Data were expressed as means ± standard deviations. Two-way-repeated measures analysis of variance (ANOVA) were used to assess the effects of groups and of the testing condition. When significant, we compared DS and CONT groups using ANOVA test (Statistica Software 8.0). We compared resting and stimulation situations using paired sample t-tests. Pearson correlations were performed to observe possible links between hormonal and autonomic functions. Significance was considered when $p<0.05$.

Results

Both groups were paired in age, body mass index, fat mass and HR_{rest}. Subjects with DS were significantly shorter than control subjects ($p<0.01$). They also had lower systolic and diastolic blood pressure than CONT ($p<0.01$), a lower weight ($p<0.01$) and a lower maximal voluntary contraction ($p<0.001$; Table 1).

Handgrip + ischemia test (Table 2)

HR was significantly increased after ischemia in CONT group only ($p<0.001$).

At rest α_{LF} was significantly lower in DS group compared to CONT ($p<0.05$) but significantly higher than CONT after ischemia ($p<0.01$). SBP ($p<0.01$), DBP ($p<0.05$) and LF_{BPV} ($p<0.01$) were significantly lower in DS compared to CONT group after ischemia (Table 3). DBP and LF_{BPV} were significantly higher after ischemia than at rest in both groups ($p<0.05$). SBP was significantly higher after ischemia than at rest in DS group only ($p<0.01$). α_{LF} was significantly lower after ischemia than at rest in CONT group only ($p<0.01$).

Norepinephrine was higher after ischemia than at rest in CONT group only ($p<0.05$).

Cold pressor test (Table3)

There was no significant HRV difference between the two groups and between the 2 conditions (Table 2).

SBP and DBP were significantly higher after immersion than at rest in both groups ($p<0.01$). LF_{BPV} was significantly lower, and α_{LF} significantly higher after immersion than at rest in CONT group only ($p<0.01$). After ischemia, LF_{BPV} ($p<0.05$) was significantly higher, and α_{LF} significantly lower in DS than in CONT group ($p<0.01$).

After immersion ACTH was significantly higher in DS than in CONT group ($p<0.001$). ACTH was significantly higher after immersion than at rest ($p<0.001$) in DS only. Norepinephrine was significantly higher after immersion in both groups ($p<0.01$ in CONT and $p<0.001$ in DS).

Head-up tilt test (Table 4)

Differences between supine and HUT position were found in the two groups. rMSSD ($p<0.001$), pNN50 ($p<0.001$) and HF ($p<0.001$) were lower in HUT position. LF ($p<0.001$), LF/HF ($p<0.001$ for CONT and $p<0.01$ for DS group) and HR ($p<0.001$) were higher in HUT position.

SBP was significantly lower in DS group compared to CONT in HUT position ($p<0.05$).

ACTH and cortisol concentrations were significantly higher in DS compared to CONT group in both conditions (Table 4).

Norepinephrine concentrations were positively related to SBP and DBP in HUT position ($r=0.74$; $p<0.05$ and $r=0.79$; $p<0.05$, Figure 2a, 2b respectively) in CONT group only.

Discussion

The aim of this work was to compare autonomic function and hormonal responses to autonomic provocation tests between DS and CONT subjects. Anthropometric characteristics of DS observed in our study correspond to the classical profile of subjects with Down syndrome, with small height and high relative weight [22,23].

Table 2. Handgrip + ischemia test responses in CONT and DS groups.

Handgrip + ischemia test Cardiac responses	CONT		DS	
	T0 Rest	**T1 post ischemia**	**T0 Rest**	**T1 post ischemia**
HR (bpm)	68 ± 11	90 ± 14^{bbb}	72 ± 10	81 ± 14
rMSSD	47.8 ± 15.8	41.7 ± 14.1	40.1 ± 20.9	39.0 ± 24.3
PNN50 (%)	24.5 ± 11.9	15.9 ± 7.2	18.4 ± 16.5	16.1 ± 17.5
LF (nu)	70.5 ± 10.0	66.7 ± 9.3	74.7 ± 8.4	69.1 ± 12.1
HF (nu)	29.5 ± 10.0	33.3 ± 9.3	25.3 ± 8.4	30.9 ± 12.1
LF/HF	2.8 ± 1.3	2.2 ± 0.7	3.4 ± 1,5	2.9 ± 2.1
Vascular responses	**T0 Rest**	**T1 post ischemia**	**T0 Rest**	**T1 post ischemia**
SBP (mmHg)	128 ± 17	155 ± 18^{bb}	108 ± 33	130 ± 13^{aa}
DBP (mmHg)	71 ± 8	89 ± 9^{bbb}	70 ± 6	79 ± 8^{ab}
LF_{BPV} (mmHg²)	6 ± 5	53 ± 12^{bb}	9 ± 5	26 ± 25^{aab}
α_{LF} (ms/mmHg)	20.53 ± 8.37	5.35 ± 1.31^{bbb}	12.43 ± 3.45^{a}	9.99 ± 5.74^{aa}
Hormonal responses	**T0 Rest**	**T1 post ischemia**	**T0 Rest**	**T1 post ischemia**
ACTH (pmol/L)	5.2 ± 2.3	8.7 ± 6.4	6.4 ± 2.7	7.2 ± 3.4
Cortisol (nmol/L)	321 ± 134	341 ± 132	359 ± 106	347 ± 111
Epinephrine (pmol/L)	226 ± 197	282 ± 253	249 ± 216	251 ± 196
Norepinephrine (pmol/L)	1851 ± 792	3327 ± 1739^{b}	2281 ± 520	2948 ± 1021

^{a}p<0.05; ^{aa}p<0.01: significant difference between CONT and DS groups; ^{b}p<0.05; ^{bb}p<0.01; ^{bbb}p<0.001: significant difference between the 2 tests conditions.. Values are means ± SD. rMSSD: root mean square of successive differences; PNN50: percentage of absolute differences in successive RR values>50 ms; LF: low frequency; HF: high frequency; LF/HF: low frequency/high frequency ratio. . SBP: systolic blood pressure; DBP: diastolic blood pressure; LFBPV: low frequency of blood pressure variability; αLF baroreflex sensitivity

Table 3. Cold pressor test responses in CONT and DS groups

Cold pressor test Cardiac responses	CONT		DS	
	T2 Rest	**T3 post immersion**	**T2 Rest**	**T3 post immersion**
HR (bpm)	68 ± 9	73 ± 13	69 ± 8	77 ± 10
rMSSD	49.8 ± 23.6	46.1 ± 25.5	42 ± 20.3	45.6 ± 22.8
PNN50 (%)	24 ± 12.9	20.2 ± 15.3	19.4 ± 16.4	18.5 ± 13.2
LF (nu)	68.4 ± 8.6	71.3 ± 11.1	69.8 ± 10.3	70.6 ±10.0
HF (nu)	31.6 ± 8.6	28.7 ± 11.1	30.2 ± 10.3	29.4 ± 12.0
LF/HF	2.4 ± 1.0	2.9 ± 1.3	2.6 ± 1.1	3.0 ± 1.7
Vascular responses	**T2 Rest**	**T3 post immersion**	**T2 Rest**	**T3 post immersion**
SBP (mmHg)	129 ± 13	144 ± 18[b]	118 ± 10[a]	132 ± 17[b]
DBP (mmHg)	72 ± 7	84 ± 9[bb]	71 ± 6	84 ± 10[bb]
LF_{BPV} (mmHg²)	6 ± 4	2 ± 3[b]	8 ± 6	14 ± 19[a]
α_{LF} (ms/mmHg)	18.93 ± 5.34	29.76 ± 10.29[bb]	15.19 ± 4.93	12.10±4.03[aaa]
Hormonal responses	**T2 Rest**	**T3 post immersion**	**T2 Rest**	**T3 post immersion**
ACTH (pmol/L)	5.2 ± 1.6	5.6 ± 1.5	5.9 ± 2.2	12.8 ± 7.6[bbaaa]
Cortisol (nmol/L)	366 ± 129	337 ± 116	342 ± 130	340 ± 121
Epinephrine (pmol/L)	218 ± 180	222 ± 137	201 ± 152	301 ± 287
Norepinephrine (pmol/L)	1970 ± 730	2930 ± 1448	2255 ± 557	3701 ± 1096[bbb]

ap<0.05; aaap<0.001: significant difference between CONT and DS groups; bp<0.05; bbp<0.01; bbbp<0.001: significant difference between the 2 tests conditions. Values are means ± SD. rMSSD: root mean square of successive differences; PNN50: percentage of absolute differences in successive RR values>50 ms; LF: low frequency; HF: high frequency; LF/HF: low frequency/high frequency ratio. . SBP: systolic blood pressure; DBP: diastolic blood pressure; LFBPV: low frequency of blood pressure variability; αLF baroreflex sensitivity

Table 4. Head-up tilt test responses in CONT and DS groups

Head-up tilt test Cardiac responses	CONT		DS	
	T4 Supine	**T5 HUT**	**T4 Supine**	**T5 HUT**
HR (bpm)	61 ± 9	84 ± 12[bbb]	66 ± 7	87 ± 10[bbb]
rMSSD	60.1 ± 18.8	28.4 ± 10.3[bbb]	52.6 ± 27.4	20.5 ± 12.2[bb]
PNN50 (%)	35.3 ± 16.6	7.4 ± 5.9[bbb]	26.9 ± 20.0	4.3 ± 6.2[bb]
LF (nu)	60.5 ± 11.5	87.7 ± 4.2[bbb]	63.5 ± 13.6	83.6 ± 6.7[bbb]
HF (nu)	39.5 ± 11.5	12.3 ± 4.2[bbb]	36.4 ± 13.6	16.4 ± 6.6[bbb]
LF/HF	2.2 ± 2.8	8.0 ± 2.8[bbb]	2.1 ± 1.2	6.0 ± 2.8[bbb]
Head-up tilt test Vascular responses	**CONT**		**DS**	
	T4 Supine	**T5 HUT**	**T4 Supine**	**T5 HUT**
SBP (mmHg)	121 ± 11	117 ± 11	104 ± 31	97 ± 29[a]
DBP (mmHg)	69 ± 6	72 ± 8	70 ± 4	70 ± 8
LF_{BPV} (mmHg²)	10 ± 8	10 ± 5	8 ± 5	12 ± 13
α_{LF} (ms/mmHg)	19.74 ± 13.57	15.85 ± 5.85	14.97 ± 5.66	10.25 ± 6.72
Head-up tilt test Hormonal responses	**CONT**		**DS**	
	T4 Supine	**T5 HUT**	**T4 Supine**	**T5 HUT**
ACTH (pmol/L)	4.4 ± 1.4	4.9 ± 1.7	7.1 ± 2.8[aa]	9.0 ± 5.3[a]
Cortisol (nmol/L)	298 ± 90	279 ± 77	403 ± 136[a]	379 ± 131[a]
Epinephrine (pmol/L)	173 ± 111	254 ± 172	172 ± 131	192 ± 138
Norepinephrine (pmol/L)	1740 ± 542	2361 ± 579[b]	2063 ± 576	2689 ± 470[b]

ap<0.05; aap<0.01: significant difference between CONT and DS groups; bp<0.05; bbp<0.01; bbbp<0.001: significant difference between the 2 tests conditions. Values are means ± SD. rMSSD: root mean square of successive differences; PNN50: percentage of absolute differences in successive RR values>50 ms; LF: low frequency; HF: high frequency; LF/HF: low frequency/high frequency ratio. . SBP: systolic blood pressure; DBP: diastolic blood pressure; LFBPV: low frequency of blood pressure variability; αLF baroreflex sensitivity

The main results showed altered vascular and hormonal responses in DS compared to CONT after an autonomic test with a lower sympathetic activation observed in handgrip + ischemia and cold pressor tests in DS group. Blood pressure was lower in DS in the 3 tests compared to CONT, with altered baroreflex sensitivity found in cold pressor and handgrip + ischemia tests. Hormonal kinetics was different between the two groups in cold pressor and HUT tests with higher ACTH and cortisol concentrations in DS.

Handgrip + ischemia test aims to investigate the role of muscle metaboreflex in neural cardiovascular regulation. In response to ischemia, metaboreceptors induce a sympathetic activation in order to maintain a sufficient blood pressure. In our study, CONT showed an appropriate HR adaptation to contraction and ischemia with a significant increase. This result was not found in DS group and is concordant with Fernhall and Otterstetter [7] who explained this result by a blunted vagal withdrawal in DS group, associated to a lower sympathetic activation.

In addition blood pressures were significantly lower in DS compared to CONT group with a lower amplitude response to contraction. Fernhall and Otterstetter [7] claimed that these low pressor values could be a function of reduced sympathetic stimulation and blunted vagal withdrawal during handgrip + ischemia test. Moreover, in our study LF_{BPV} values were lower in DS compared to CONT during contraction as observed in several studies [7,8]. These values of DS group provide

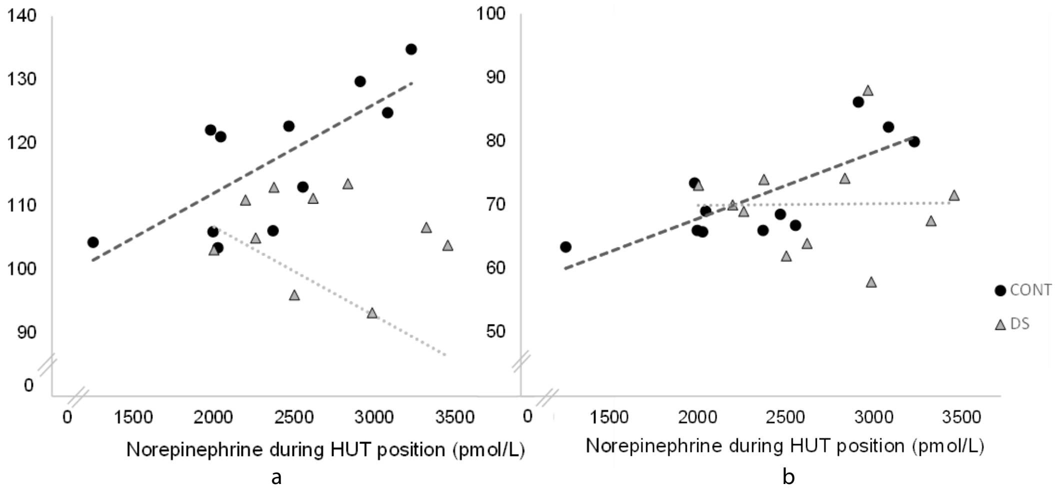

Figure 2. Relation between hormonal and vascular responses to head-up tilt test position.

HUT: head-up tilt; LF: low frequency: SBP: systolic blood pressure; DBP: diastolic blood pressure

2a: correlation between systolic blood pressure and norepinephrine concentration in HUT position r=0.74; p<0.05

2b: correlation between diastolic blood pressure and norepinephrine concentration in HUT position r=0.79; p<0.05

evidence of a reduced sympathetic activity during handgrip test as LF_{BPV} is a measure of vascular sympathetic modulation [18,19].

The low baroreflex delta observed in DS could reflect an inadequate autonomic modulation in response to handgrip + ischemia, arguing for a dysautonomia in this population. In fact CONT group showed a wide decrease of baroreflex sensitivity index during contraction whereas in DS only a slight difference (by 24% vs 73% for CONT) was found arguing in favor of an altered baroreflex sensitivity. In numerous studies baroreflex decreases during isometric exercise, caused by a reset of barosensitivity to a higher set-point during static contraction [24,25]. As this baroreflex modulation permits to reduce vagal activity in response to mechanoreceptors [25,26], we can assume that in DS this vagal modulation is not effective and could explain the lower heart rate and blood pressure.

Furthermore, hormonal adaptations confirmed these observations. In CONT there was an increase of norepinephrine following the handgrip + ischemia test whereas it did not occur in DS group (+79% in CONT vs +29% in DS). This hormonal adaptation in CONT reflects a sympathetic adaptation due to stress of contraction and ischemia, which could not be found in DS.

In another hand, the common hypotonia in subjects with DS can limit to sustain a constant contraction during 2 minutes in Down syndrome group. As a result, it could induce a lower amplitude of autonomic response to this test. That was the case in our study with a lower maximal voluntary contraction in DS and difficulties to maintain a static contraction.

The **cold pressor test** aims to increase systolic blood pressure during immersion [27,28]. In our study SBP and DBP increased in both groups attesting of an appropriate pressor response to immersion. Nevertheless, CONT group showed wider increased values of LF_{BPV} compared to DS (173% for CONT *vs* -45% for DS). Fernhall and Otterstetter [7] observed similar results and suggested an increase of vascular sympathetic activity in DS associated with a blunted baroreflex sensitivity. Lower baroreflex sensitivity index in DS confirms this alteration as DS did not adjust this index in immersion while CONT presented a significant increase.

Surprisingly, in DS we observed that ACTH concentration was significantly higher than in control subjects during immersion and remained elevated 20 minutes after rest. Because this increase was not accompanied by a cortisol increase, a corticotropic dysfunction should be considered. Murdoch *et al.* [29] have shown a blunted cortisol response to a Synacthen® test in population with DS, suggesting a cortisol deficiency. In our study this phenomenon seemed to be similar. If ACTH response was effective after stimulation (+54%, p<0.01), the cortisol gland did not respond by an appropriate cortisol secretion. This observation was exclusive in DS group and could corroborate the cortical gland deficiency proposed by Murdoch *et al.* [29]. Moreover norepinephrine concentrations were higher after immersion in DS, and this effect was not observed in CONT group. This observation can reflect a very high stress felt by the participants with DS. Indeed, the hand-immersion into 6°C water has been very difficult and painful for the DS participants. Therefore, the local phenomenon of vasoconstriction mediated by norepinephrine could be higher in DS and be associated to pain signs.

The head-up tilt test aims to increase heart rate in response to orthostatic stress and to induce a blood redistribution to the lower limbs, with a decrease of venous return and a reduction in the diameter of the left ventricle. These adaptations stimulate the baroreceptors which are sensitive to pressure variations and their stimulation activates the vasomotor center, resulting in catecholamines secretion by sympathetic nervous system [30].

In our study we can observe an appropriate cardiac adaptation to HUT test in both groups, with no significant differences between the two groups. This is in line with the cardiac autonomic modulation observed with HUT in a range of different populations [31-34]. An adaptation of parasympathetic tone on HUT stimulation was evidenced by the reduced rMSSD values and HF spectrum (Table 4). This demonstrates that the HUT maneuver adequately induced vagal withdrawal and subsequently led to HR increase. This HR normal adaptation in DS was also described by others [34,35] who found no differences in HUT test between DS and control groups.

While this adaptation occurred in both groups, the hormonal adaptation was different. In participants with DS, the ACTH and cortisol concentrations were higher in supine and HUT position, and ACTH increased by 20% ($p<0.05$) between supine and tilted positions whereas it increased by 10% (not significant) in the control group. Vascular response was also different in DS group with lower systolic blood pressure in HUT position. This response to head-up tilt test was described in many studies with frequent hypotension in DS, and a lower blood pressure response to stress like exercise, handgrip or orthostatic stress [7,24,36]. Agiovlasitis *et al.* [37] observed lower blood pressure and no HR differences between DS and control during head-up tilt test. Baroreflex sensitivity was expected to decrease in both groups as observed in other studies [34,35,37]. In our results, α_{LF} values differed significantly between groups and between the test conditions. This inconsistence could be explained by different methodological approaches. Iellamo *et al.* [34,35] and Agiovlastitis *et al.* [37] used a sequence method in time domain to calculate baroreflex sensitivity whereas we used an open-Loop technique, in accordance to Barbieri *et al.* [20].

Finally, relation between norepinephrine and blood pressures in CONT group but not in DS group suggest that increase of norepinephrine is associated with a higher sympathetic activity. This result is expected in general population and is not observed in DS group. In fact it is established that an increase of norepinephrine induces a higher systolic and diastolic blood pressures [38]. In DS group this increase is not associated with an appropriate pressive response, suggesting a blunted vascular response. Thus, despite normal HR and HRV changes, yet hormonal secretions and blood pressure adaptations provide evidence of an autonomic dysfunction in persons with DS as these parameters are mediated by autonomic nervous system.

This work aimed to compare autonomic function and hormonal responses to autonomic provocation tests between DS and CONT subjects. A highlighted relation between autonomic adaptations and hormonal responses made the result of the study original.

A blunted baroreflex sensitivity and a lower sympathetic activation were observed in 2 of the 3 tests in DS group. The head-up tilt test results showed a pertinent experimental test as it showed an appropriate autonomic response in CONT whereas there was a mismatch in participants with DS concerning hormonal and vascular responses. Cold pressor test, used to stimulate the sympathetic system, seemed to provoke lower responses in DS and to induce defense reactions that could alter results. Handgrip test was interesting to complete explorations and to verify baroreflex alterations.

Limitations

Data treatments demand expertise and time. Moreover, HRV and BPV are indirect measures of autonomic nervous system and a direct stimulation of autonomic nerves could be more accurate. Moderate number of subjects constituted a second limit in this work as physiological responses were not homogenous in DS group. As the men-only recruitment was a limit, further investigations are needed to generalize results to women.

Conclusion

To our knowledge this is the first study assessing concurrently hormonal and autonomic responses in population with DS. In addition, this work provided complementary knowledge on HRV utilization in multiple situations that aim to stimulate autonomic nervous system.

Investigation of the cardiovascular system and its regulation mechanisms in physiological conditions demands adapted tools. In our work a special attention was given to supply a non-invasive technique. HRV utilization responds to both criteria: it is a pertinent tool to better understand responses of participants with DS when they undergo stimulation tests, and it allows to identify possible cardiovascular limits to exercise or other stress. In addition, head-up tilt test is widely used in physiology to describe neurovegetative and cardiovascular reflex thanks to its simplicity, reproducibility and the availability of numerous results concerning these adaptations [39-43]. The crossing of the three test results allowed to get an accurate diagnosis respecting Ewing *et al.* [44] classifications. Moreover, relations between blood pressure and hormonal variables supply complementary information that reinforce HRV observations.

Dysautonomia in persons with DS could induce maladaptation to stress or to physical exercise with inadequate heart rate, blood pressure and endocrine responses. An identification of these autonomic dysfunctions will permit to propose a better clinical management in population with Down syndrome.

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