

FOLIA MEDICA CRACOVIENSIA

Vol. LIX, 4, 2019: 95–110

PL ISSN 0015-5616

DOI: 10.24425/fmc.2019.131383

Cardiovascular response to different types of acute stress stimulations

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Abstract: **Introduction:** Stress is an ubiquitous phenomenon in the modern world and one of the major risk factors for cardiovascular disease. The aim of our study was to evaluate the effect of various acute stress stimuli on autonomic nervous system (ANS) activity, assessed on the basis of heart rate (HRV) and blood pressure (BPV) variability analysis.

Materials and Methods: The study included 15 healthy volunteers: 9 women, 6 men aged 20–30 years (23.3 ± 1.8). ANS activity was assessed by HRV and BPV measurement using Task Force Monitor 3040 (CNSystems, Austria). ECG registration and Blood Pressure (BP) measurement was done 10 minutes at rest, 10 minutes after the stress stimulus (sound signal, acoustic startle, frequency 1100 Hz, duration 0.5 sec, at the intensity 95 dB) and 10 minutes after the cold pressor test. The cold pressor test (CPT) was done by placing the person's hand by wrist in ice water ($0-4^{\circ}\text{C}$) for 120 s.

Results: Every kind of stress stimulation (acoustic startle; the CPT) caused changes of HRV indicator values. The time domain HRV analysis parameters (pNN50, RMSSD) decreased after acoustic stress and the CPT, but were significantly lower after the CPT. In frequency domain HRV analysis, significant differences were observed only after the CPT: (LF-RRI 921.23 ms^2 vs. 700.09 ms^2 ; $p = 0.009$ and HF-RRI 820.75 ms^2 vs. 659.52 ms^2 ; $p = 0.002$). The decrease of LF-RRI and HF-RRI value after the CPT was significantly higher than after the acoustic startle (LF-RRI 34% vs. 0.4%, $p = 0.022$; HF-RRI 19.7% vs. 7% ms^2 , $p = 0.011$). The decreased value of the LF and HF components of HRV analysis are indicative of sympathetic activation. Nonlinear analysis of HRV indicated a significant decrease in the Poincare plot SD1 ($p = 0.039$) and an increase of DFA α_2 ($p = 0.001$) in response to the CPT stress stimulation. The systolic BPV parameter LF/HF-sBP increased significantly after the CPT (2.84 vs. 3.31; $p = 0.019$) and was higher than after the acoustic startle (3.31 vs. 3.06; $p = 0.035$). Significantly higher values of diastolic BP (67.17 ± 8.10 vs. $69.65 \pm 9.94 \text{ mmHg}$, $p = 0.038$) and median BP (83.39 ± 8.65 vs. $85.30 \pm 10.20 \text{ mmHg}$, $p = 0.039$) were observed in the CPT group than in the acoustic startle group.

Conclusions: The Cold Pressor Test has a greater stimulatory effect on the sympathetic autonomic system in comparison to the unexpected acoustic startle stress. Regardless of whether the stimulation originates from the central nervous system (acoustic startle) or the peripheral nervous system (CPT), the final response is demonstrated by an increase in the low frequency components of blood pressure variability and a decrease in the low and high frequency components of heart rate variability.

Key words: autonomic nervous system, acoustic startle, cold pressor test, heart rate variability, blood pressure variability.

Introduction

Stress is an omnipresent phenomenon in everyday life. The response of the cardiovascular system (increase in blood pressure, heart rate, peripheral resistance) to repeated acute stress can be considered a key pathophysiological mechanism in various cardiovascular conditions, including congestive heart failure, essential hypertension, disorders of postural circulatory control causing syncope, and “psychogenic cardiovascular disease,” that is, heart disease attributable to mental stress and psychiatric illness. These abnormalities involve persistent activation of sympathetic outflow to the heart and kidneys in heart failure and hypertension [1–8], either intermittent or ongoing cardiac sympathetic activation in psychogenic heart disease [9–11], and faulty sympathetic circulatory reflexes in the disorders of postural circulatory control [12–15].

Acute distress responses are executed by the autonomic nervous system (ANS), which operates by involuntary reactions. The ANS is comprised of two antagonistic components: the sympathetic nervous system, and the parasympathetic nervous system, which enables the restoration of homeostasis via delayed responses and which dominates in quiet resting activities (*rest-and-digest response*). The ANS thus plays a crucial role in the maintenance of homeostasis [16]. The central nervous system (CNS), such as cortical centers (insula), hypothalamus, amygdala, pons, and medulla regulates the ANS function.

Response to acute stress begins in the brain. The moment when the body’s receptors and sensory organs (e.g. tactile, temperature, pain receptors as well as the eyes and auditory organs) recognize a potential threat in the environment, the stimulus is transmitted through afferent tracts to the thalamus, and thus by subcortical afferent connections towards the amygdala, which is a part of the limbic system located in the temporal lobes. The amygdala interprets the threatening images and sounds and then, apart from generating negative emotions or aggression, it also generates stress reactions by stimulating the sympathetic nervous system via efferent subcortical projections to the hypothalamus and brainstem, where centers responsible for vegetative reactions, such as breathing, blood pressure, heartbeat, and the dilation

or constriction of blood vessels and bronchioles, are situated. The hypothalamus thus regulates and integrates a number of metabolic processes, as well as the co-functioning of the autonomic nervous system and the central nervous system [17].

The signal then passes through sympathetic nerves which synapses are at the medulla of the adrenal gland. Epinephrine (adrenaline), often called the stress hormone, is secreted by medullary chromaffin cells into the blood stream. The hormone interacts with adrenergic receptors dispersed throughout internal organs and causes a range of physiological responses of effector organs, such as pupillary dilation, piloerection, bronchial tubes dilation, increased heart rate and contractility, and as increased activity of idiopathic pacemakers in the ventricles. It also increases blood pressure by constriction of blood vessels in the skin and mucosa, cerebral, renal, and abdominal viscera — blood flow is then diverted to skeletal and cardiac muscle via dilation of skeletal and coronary arteries [17].

The Hennes and Brown Test (cold pressor test, CPT) can be performed in the course of a cardiological workup to assess autonomic nervous system and also left ventricle function [18, 19]. This elicits an instantaneous local and generalized vasoconstriction in the skin and the skeletal muscles, which is not only a direct effect of cold on the local skin vessels, but also a result of pain-activating spinal cord and hypothalamic reflexes. The heart rate increases to a peak value within the first 30 s and returns to baseline level within one minute. Due to an increase in total peripheral resistance, arterial pressure increases, with peak values in the second minute of the test. The pressor response is strongly correlated with increased muscle sympathetic neural activity.

Heart rate variability (HRV) is assessed by measuring beat to beat changes in heart rate [20]. HRV is a method of monitoring the risk of mortality from cardiac arrhythmias and other dynamical diseases [21]. In addition to the most frequently used time-domain, frequency-domain, and geometric methods of analysis of heart rate variability, the non-linear method has been proposed. The most commonly utilized non-linear methods are the Poincare plot, detrended fluctuation analysis, correlation dimension, recurrence plot, approximate entropy, and sample entropy.

Under healthy conditions, the normal cardiac (sinus) interbeat interval fluctuates in a complex manner [22]. Applying HRV analysis based on methods of non-linear dynamics thus yields valuable information. The main inputs are the sympathetic and the parasympathetic nervous system and humoral factors. Other contributing factors are the baroreflex, thermoregulation, hormones, sleep-wake cycle, meals, physical activity, and stress [23].

Non-linear methods have been applied to distinguish the autonomic contributions between HRV modulations. HRV is the result of a complex regulatory system related to the electrical depolarization of cardiac cells, which is primarily regulated by the autonomic nervous system, the mechanical and functional properties of cardiac cells, and electrolytes acting on the refractory period of the action potential in the cardiac

cells. Non-linear methods are useful tools characterizing these properties of the cardiac regulatory system quantitatively from one of its measures, that is, the heart rate [24]. Certain pathological conditions such as myocardial infarction, diabetes mellitus, and aging are defined by the loss of complexity in the dynamics of the heart rate regulatory system [25]. The most important role of non-linear HRV indices is to improve the stratification of patients at high risk of cardiovascular death [25–27]. Therefore, one of the key diagnostic advantages of non-linear HRV indices is that they provide unique information about a given patient's cardiological status.

Aim

The aim of the study was to analyze the effect of two different types of acute stress on the activity of the autonomic nervous system and the hemodynamic parameters of the cardiovascular system.

Material and Methods

The study included 15 healthy volunteers with mean age 23.3 ± 1.8 ; 9 women and 6 men.

In this study we excluded persons with diabetes mellitus, obesity ($BMI \geq 30 \text{ kg/m}^2$), cardiovascular diseases (hypertension, coronary artery disease, valvular heart disease, cardiac arrhythmias), tobacco smoking, intake of medications that may interfere with autonomic system activity, previous surgeries, as well as any pathologies of the GI tract (e.g. inflammatory bowel disease), gynecological systems, or chronic diseases.

The study protocol

All subjects were asked to fast for at least 12 hours prior to the examination and to discontinue any medications with a known effect on the ANS three days before the study. The examination took place at room temperature, in a quiet relaxed atmosphere. The evaluation of ANS activity comprised of:

1. 10 min heart rate variability (HRV) recording at rest
2. HRV recording 10 min following exposure to a stressor (sound signal)
3. HRV recording 10 min following cold pressor test.

Assessment of the autonomic nervous system

ANS activity was assessed by heart rate variability and blood pressure variability (BPV) measured with the Task Force Monitor 3040 (CNSystems, Austria) and analyzed with Task Force V Monitor 2.2 software. ECG signals were registered during

10-minute intervals: 10 minutes at rest, 10 minutes after exposure to a stressor (sound signal), and 10 minutes after the cold pressor test.

The following **frequency domain analysis HRV parameters** were evaluated: LF (component of the low-frequency range, 0.04–0.15 Hz, modulated by both the sympathetic and parasympathetic nervous system and associated with baroreceptor activity), HF (component of the high-frequency range, 0.15–0.4 Hz, modulated by the parasympathetic nervous system, associated with respiration and blood pressure changes), and LF/HF ratio, reflecting interactions of both types of autonomic modulation and normalized components, LFnu [$LF/(TP-VLF)*100$] and HFnu [$HF/(TP-VLF)*100$] [19, 20].

The following **frequency domain BPV indices** were calculated: PSD, VLF, HF as like in HRV analysis and mid frequency (0.1 Hz) oscillations (MF), i.e. Mayer waves, and low frequency (<0.1 Hz) oscillations (LF) — depending on many physiological phenomena. MF and LF were analyzed together as the low frequency spectrum controlled by vascular innervation and modulated by autonomic activity.

Additionally, several **time domain analysis HRV parameters** were calculated from the experimental data: SDNN (standard deviation of all NN intervals), RMSSD (the square root of the mean of the sum of the squares of differences between adjacent NN intervals), pNN50 (number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording divided by the total number of all NN intervals). These parameters were calculated using KubiosPro2.0 software (Kuopio, Finland).

The following **nonlinear HRV analysis indices** were calculated using KubiosPro2.0 software: recurrency (%REC), determinism (%DET), DFA α 1 (short-term fractal exponent of Detrended Fluctuation Analysis that correspond to a period of 4–16 RRi) and DFA α 2 (long-term fractal exponent of DFA that correspond to a period of 16–64 RRi), MSE (the slope from the multiscale entropy (MSE) measured with two different entropy estimators (the approximate entropy (ApEn) and the sample entropy (SampEn)) and Poincare plot. The entropy rate measures the increase of sequence entropy when an extra sample is added. The entropy rate drops when the sequence grows, according to a regular and predictable pattern. Conversely, a constant entropy rate suggests that each new sample added is not completely predictable. The entropy rate is often simplified when referring to HRV analysis as ‘entropy’.

The Poincare plot method of RR intervals analysis allows us to visualize instantaneous relationships between current RR (RR_i) and following RR (RR_{i+1}) interval. Each data point represents a pair of successive beats, the x-axis (abscissa) is the current RR interval, while the y-axis (ordinate) is the next RR interval. In addition, all the points for which the duration of the current and the next RR interval is the same ($RR_i = RR_{i+1}$), the corresponding scatters fall exactly on the line of equality. All RR intervals associated with the decrease of heart rate are under the identity line, and RR intervals of increased heart rate will be located above the line. HRV is quantified

by fitting mathematically defined geometric shapes such as an imaginary eclipse to the data. To characterize the scatter points along the two axes of the formed ellipse, two descriptors, SD1 and SD2, are used, wherein SD1 is characterized by distribution of points across the line of identity, while SD2 along this line. Pearson's correlation coefficient r describes the relationship between the pairs of values of abscissae and ordinates, which in the case of RR intervals is equivalent to the relationship between successive RRs. SD1 describes the short-term and SD2 long-term heart rate variability. The coefficient r , however, determines the balance between SD1 and SD2, that is, between short-term and long-term variation [28].

Hemodynamic indices were determined using the cardioimpedance method programmed into the Task Force Monitor 3040 (CNSystems, Austria). The following hemodynamic parameters were measured: HR: heart rate; sBP: systolic blood pressure; dBP: diastolic blood pressure; mBP: mean blood pressure (beat to beat); SV: stroke volume; SI: stroke index; CO: cardiac output; CI: cardiac index; TPR: total peripheral resistance; TPRI: total peripheral resistance index; IC: index of contractility; ACI: acceleration index; PEP: pre-ejection period; LVET: left ventricular ejection time; STR: systolic time ratio; ER: ejection rate; LVWI: left ventricular work index; MSER: mean systolic ejection rate; REP: rapid ejection period; HI: Heather index; RZ: R-dZmax time; TAC: total arterial compliance; BRS: Baroreceptor Reflex Sensitivity (spontaneous activity of baroreceptors determined using the "sequence method" which detects rising sequences, i.e. an increase in systolic blood pressure and longer R-R intervals, and falling sequences, i.e. a decrease in systolic blood pressure and shorter R-R intervals, from continuous beat-to-beat time series of R-R intervals and systolic blood pressure recordings); and BEI: Baroreceptor Effectiveness Index (ratio of baroreceptor sequences/events for lags 0, 1 and 2 as related to the number of BP ramps).

Acoustic stressor

The sound stressor was generated using a standardized sound creating device. The characteristics of the sound stressor were: 1100 Hz frequency, 0.5 second duration, and intensity of 100 dB. The device was designed to produce an unexpected sound stressor (acoustic startle) affecting the subject. It was placed 2 meters from the subject.

Cold Pressor Test

The Cold Pressor Test was performed by placing the subject's hand up to the wrist in ice water (0–4°C) for 120 s. The subject was examined in a horizontal position, with continuously HR and beat-to-beat BP monitoring. The response to the test was normal (indicating proper ANS functioning) when the subject's BP and HR profiles demonstrated a two-phase pattern: the first phase characterized by an increase in

HR in the first 30s, and the second phase characterized by an increase in BP and peripheral resistance in the second minute of the test. The abnormal response to the test (indicating ANS dysfunction) was when the increase in BP was lower than 15 mmHg.

Ethics

The study was approved by Local Bioethics Committee at the Jagiellonian University (decisions no. 122.6120.26.2017 and no. KBET/148/B/2012). All enrolled subjects were provided with information on the study objectives and gave their written informed consent for the participation in the experiment beforehand. All procedures were compliant with the Declaration of Helsinki.

Statistical analysis

TIBCO Statistica for Windows, version 13.3 PL (TIBCO Software Inc., USA, Jagiellonian University license) was used for database management and statistical analysis. Normal distribution of quantitative variables was verified with the Shapiro–Wilk test and the equality of variances was checked with the Levene test. Their statistical characteristics were presented as means and standard deviations (SD). Variables that did not satisfy the criteria of normality were presented as median (Me) and maximum and minimum values (min-max). Depending on the distribution type, the significance of intragroup differences was verified with a parametric two-way ANOVA test that has been performed to examine the differences between variables when normality was present. The Friedman two-way analysis of variance (ANOVA) by rank test with post-hoc ANOVA Friedman test was done to evaluate the differences between all the investigated groups in variables without normal distribution. The power of associations between the values of HRV, BPV parameters and cardiovascular hemodynamic indices was estimated on the basis of Spearman's coefficients of rank correlation. The results of all tests were considered statistically significant with $p < 0.05$.

Results

Linear and nonlinear HRV analysis

Every kind of stress stimulation (acoustic startle; the CPT) caused changed values of HRV indicators, but only after the CPT was such change statistically significant (LF-RRI 921.23 ms^2 vs. 700.09 ms^2 ; $p = 0.009$, HF-RRI 820.75 ms^2 vs. 659.52 ms^2 ; $p = 0.002$). Also the decrease of LF-RRI and HF-RRI value after the CPT was

significantly higher than after the acoustic startle (LF-RRI 34% vs. 0.4%, $p = 0.022$; HF-RRI 19.7% vs. 7% ms^2 , $p = 0.011$) (Table 1).

Table 1. The differences in linear analysis HRV (time and spectral) indices between rest, response to the cold pressure test and the acoustic startle.

Parameters	REST [Me (min-max)] or [Mean \pm SD]	ACOUSTIC STARTLE [Me (min-max)] or [Mean \pm SD]	CPT [Me (min-max)] or [Mean \pm SD]	p^*	$p^\#$	$p^\$$
SDNN [ms]	97.18 \pm 38.93	80.82 \pm 37.20	85.33 \pm 30.04	0.097	0.062	0.679
pNN50 [%]	40.03 \pm 27.01	42.03 \pm 25.73	37.40 \pm 22.77	0.836	0.026	0.006
RMSSD [ms]	80.38 \pm 54.94	79.67 \pm 49.44	68.51 \pm 38.33	0.062	0.021	0.023
LFnu-RRI [%]	47.32 \pm 20.85	47.42 \pm 19.14	49.00 \pm 16.59	0.245	0.084	0.433
HFnu-RRI [%]	52.67 \pm 20.85	52.58 \pm 19.14	50.99 \pm 16.59	0.245	0.084	0.433
VLF-RRI [ms^2]	419.75 (188.44–7230.75)	515.03 (71.68–59843.38)	1053.21 (138.56–29542.51)	0.397	0.778	0.048
LF-RRI [ms^2]	921.23 (242.08–3182.44)	917.98 (358.82–3523.48)	700.09 (243.17–2818.18)	0.975	0.009	0.022
HF-RRI [ms^2]	820.75 (139.69–12223.50)	882.71 (152.82–13002.36)	659.52 (184.15–7080.93)	0.363	0.002	0.011
PSD-RRI [ms^2]	4820.25 (732.52–57949.27)	2736.18 (1112.55–60676.21)	3104.21 (678.29–29993.88)	0.470	0.109	0.433
LF/HF-RRI	0.85 (0.21–6.88)	0.78 (0.27–4.11)	1.23 (0.34–2.86)	0.074	0.245	0.875

p — Friedman ANOVA with post-hoc (* — rest vs. acoustic startle, # — rest vs. CPT, \$ — acoustic startle vs. CPT)

The decreased values of the LF and HF components of HRV analysis indicates sympathetic overactivity.

The time domain HRV analysis parameters: SDNN, pNN50, RMSSD significantly decreased after the CPT.

Nonlinear analysis of HRV indicated in response to CPT stress stimulations a significant decrease of the Poincare plot SD1 ($p = 0.039$) and an increase of DFA α 2 ($p = 0.001$). Acoustic startle stress caused an increase of ApEn ($p = 0.039$) and SampleEn ($p = 0.07$). The exact values of these indices are shown in Table 2.

Table 2. The differences in nonlinear analysis HRV (fractal) indices between rest, response to the acoustic startle and the cold pressure test.

Parameters	REST [Mean ± SD]	ACOUSTIC STARTLE [Mean ± SD]	CPT [Mean ± SD]	p*	p [#]	p ^{\$}
Poicare plot SD1	56.93 ± 38.93	56.44 ± 35.04	48.53 ± 27.15	0.836	0.039	0.302
Poicare plot SD2	122.89 ± 45.29	106.42 ± 33.52	109.81 ± 35.15	0.179	0.803	0.453
%REC	33.75 ± 14.85	32.79 ± 13.69	31.11 ± 8.02	0.438	0.803	0.803
%DET	97.64 ± 1.91	97.47 ± 1.67	97.75 ± 1.27	0.438	0.803	0.803
ShanEn	3.23 ± 0.52	3.14 ± 0.38	3.15 ± 0.35	0.469	0.453	0.803
ApEn	1.09 ± 0.09	1.14 ± 0.09	1.11 ± 0.09	0.039	0.453	0.803
SampleEn	1.51 ± 0.35	1.63 ± 0.26	1.58 ± 0.35	0.070	0.803	0.803
DFAα1	0.97 ± 0.28	0.97 ± 0.29	0.96 ± 0.23	0.535	0.803	0.803
DFAα2	0.93 ± 0.21	0.85 ± 0.18	1.04 ± 0.14	0.301	0.001	0.211

p — Friedman ANOVA with post-hoc (* — rest vs. acoustic startle, # — rest vs. CPT, \$ — acoustic startle vs. CPT)

BPV analysis

Parameters of systolic and diastolic BPV (PSD-dBP, LF-sBP and PSD-sBP) were significantly lower after the CPT. HF-dBP was the only BPV parameter that was significantly lower after the acoustic startle ($p = 0.001$). The systolic BPV parameter LF/HF-sBP increased significantly after the CTP (2.84 vs. 3.31; $p = 0.019$) and was higher than after the acoustic startle (3.31 vs. 3.06; $p = 0.035$). The exact BPV parameters are shown in Table 3.

Cardiovascular hemodynamics parameters analysis

Significant differences in HR as well as systolic parameters were not observed. Diastolic and mean BP parameters were higher in the CPT group, but statistically significant differences were observed only in relation to the acoustic startle group. Analysis of baroreceptor sensitivity revealed lower values of BRS in the CPT patients than in the acoustic startle group. These results are correlated with sympathetic overactivity in the CPT group. However, it was not statistically significant. Differences in these parameters between the rest and CPT groups were not observed, and TPRI was nearly the same in all three groups. Cardiac index was higher in the acoustic startle group than in rest and CPT groups, but it was not statistically significant. The results are shown in Table 4.

Table 3. The differences in spectral analysis systolic and diastolic BPV indices between rest, response to the cold pressure test and the acoustic startle. dBP — diastolic blood pressure; sBP — systolic blood pressure.

Parameters	REST [Me (min-max)] or [Mean ± SD]	ACOUSTIC STARTLE [Me (min-max)] or [Mean ± SD]	CPT [Me (min-max)] or [Mean ± SD]	p*	p [#]	p ^{\$}
LFnu-dBP [%]	50.11 ± 13.46	49.16 ± 12.87	48.62 ± 11.85	0.650	0.427	0.650
HFnu-dBP [%]	16.78 ± 11.23	17.47 ± 11.01	17.36 ± 11.16	0.334	0.281	0.691
VLF-dBP [ms ²]	1.10 (0.28–4.48)	1.39 (0.28–4.03)	1.17 (0.22–3.58)	0.820	0.334	0.173
LF-dBP [ms ²]	2.08 (0.94–3.97)	2.33 (0.71–3.72)	1.98 (0.63–3.33)	0.955	0.053	0.017
HF-dBP [ms ²]	0.75 (0.08–2.90)	0.69 (0.08–3.48)	0.80 (0.08–3.84)	0.001	0.691	0.001
PSD-dBP [ms ²]	4.26 (1.29–9.92)	4.24 (1.08–10.03)	3.90 (0.94–9.52)	0.955	0.027	0.008
LF/HF-dBP	3.36 (0.69–16.44)	3.10 (0.65–16.52)	2.94 (0.73–18.24)	0.363	0.496	0.001
LFnu-sBP [%]	43.44 ± 10.65	42.33 ± 10.06	41.22 ± 9.64	0.875	0.510	0.433
HFnu-sBP [%]	16.95 ± 10.22	15.86 ± 10.87	15.71 ± 8.38	0.826	0.925	0.638
VLF-sBP [ms ²]	1.52 (0.29–9.79)	2.05 (0.72–7.78)	1.92 (0.68–6.06)	0.975	0.177	0.084
LF-sBP [ms ²]	2.50 (8.71–9.31)	2.23 (0.75–5.75)	1.73 (0.93–3.52)	0.221	0.019	0.002
HF-sBP [ms ²]	0.56 (0.33–5.94)	0.59 (0.19–4.64)	0.62 (0.16–2.24)	0.041	0.140	0.124
PSD-sBP [ms ²]	4.51 (1.51–25.05)	4.36 (1.83–13.84)	4.04 (2.49–10.71)	0.272	0.026	0.004
LF/HF-sBP	2.84 (0.77–6.01)	3.06 (0.69–10.56)	3.31 (0.80–8.60)	0.510	0.019	0.035

p — Friedman ANOVA with post-hoc (* — rest vs. acoustic startle, # — rest vs. CPT, \$ — acoustic startle vs. CPT)

Table 4. Differences in hemodynamics indices between rest, response to the cold pressure test and the acoustic startle.

Parameters	REST [Mean ± SD]	ACOUSTIC STARTLE [Mean ± SD]	CPT [Mean ± SD]	p*	p [#]	p ^{\$}
HR [bpm]	65.41 ± 10.11	65.74 ± 9.05	66.63 ± 9.06	0.453	0.453	0.803
sBP [mm Hg]	107.98 ± 10.63	109.13 ± 12.02	108.91 ± 12.42	0.802	0.605	0.121
dBP [mm Hg]	67.48 ± 6.83	67.17 ± 8.10	69.65 ± 9.94	0.802	0.041	0.038
mBP [mm Hg]	83.40 ± 7.20	83.39 ± 8.65	85.30 ± 10.20	0.803	0.041	0.039
BRS [ms/mm Hg]	32.56 ± 19.51	36.23 ± 18.93	32.18 ± 15.47	0.569	0.609	0.140
BEI [%]	74.50 ± 10.70	75.90 ± 9.96	75.92 ± 9.02	0.408	0.460	0.691
TPRI [dyne*s*m ² /cm ⁵]	2279.85 ± 743.78	2285.80 ± 811.14	2280.44 ± 621.27	0.999	0.989	0.999
CI [l/min*m]	3.08 ± 0.87	3.28 ± 1.64	3.14 ± 1.07	0.546	0.752	0.989
LVET [ms]	304.69 ± 17.91	301.78 ± 16.86	301.37 ± 16.45	0.453	0.453	0.803
LMVI [mmHg*l/min/m]	3.48 ± 1.05	3.50 ± 1.77	3.44 ± 1.18	0.989	0.989	0.989
TAC [ml/mm Hg]	1.99 ± 0.38	1.96 ± 0.78	2.00 ± 0.59	0.546	0.504	0.989

p — ANOVA or Friedman ANOVA with post-hoc test (* — rest vs. acoustic startle, # — rest vs. CPT, \$ — acoustic startle vs. CPT)

The response to the test was normal in all investigated subjects. In the first 30s an increase in HR was noted. In the second minute of the test an increase in BP and peripheral resistance was observed. They demonstrated a two-phase response.

Discussion

The aim of this study was to demonstrate differences in response to two different stressor stimuli (acoustic startle and cold stress test) in a group of healthy volunteers. We analyzed the influence on autonomic nervous system activity based on linear and nonlinear analysis of HRV, the frequency domain analysis of BPV, baroreceptor sensitivity and correlation with cardiovascular system response (hemodynamic parameters) by stress. According to our knowledge, this is the first study to analyse the relationship and differences between these two ANS-activating tests. The principal findings of our study can be summarized as follows:

1. Linear HRV analysis parameters (LF, HF, SDNN, pNN50, RMSSD) were significantly lower in response to the stress stimulations. Furthermore, significant increases of the LFnu parameter were observed after CPT.
2. Nonlinear analysis of HRV indicated a significant decrease of Poincare plot SD1 and increase of DFA α 2 in response to CPT stress stimulations. Acoustic startle stress caused an increase in ApEn and SampleEn.
3. Comparison the ANS responses to these two types of stimulation revealed that changes of HRV and BPV indices were higher in the CPT group than in the acoustic startle group.
4. Hemodynamic indices confirmed sympathetic activation after both stimuli but the response to the CPT was significantly higher than the response to the acoustic startle.

In the Cold Pressor Test, the proper physiologic response is an increase in blood pressure by 16 mmHg, which is the result of sympathetic nervous system stimulation [29]. A change in pressure of less than 10 mmHg is interpreted as inadequate and permits the diagnosis of autonomic nervous system dysfunction [29]. The perception of temperature change is done by Krause and Ruffini corpuscles and relays the afferent sensory signals to the centers of the medulla oblongata, the pons and the diencephalon, where the descending reaction of the sympathetic nervous system is activated. The activation in healthy subjects causes vasoconstriction and a subsequent increase in systolic as well as diastolic blood pressure, 20–15 and 15–10 mmHg respectively [29–31]. Another effect is the increase in HR [32] and an increase in left ventricular filling pressure, but no increase in SV is observed, as a result of the increase in total peripheral resistance by vasoconstriction which increases afterload [30].

Weise *et al.* [30] demonstrated that after the CPT, LF components of the heart rate and blood pressure rise. Similar results were acquired by Sanchez-Gonzales *et al.* [33], who used the CPT to evaluate associations between sympathetic vasomotor tone and depression symptoms in young females. In both groups (healthy female volunteers and women with depression) the CPT activated the sympathetic nervous system, with a significant rise in the LF components of heart rate and blood pressure. Ghiazi *et al.* [34] further proved that the CPT activates the LF components of HRV and BPV analysis. They suggested combining the HRV tests with time-varying estimates from electrodermal activity (EDA) — the study showed that this combination could increase the statistical discriminant power more than when using only the HRV itself. Our observations only partly confirm the results of these previous studies. In our study, some LF components of the HRV increased (LFnu, VLF-RRI) but some decreased (in the case of LF-RRI, significantly). The decrease of HRV parameters (LF and HF) is indicators of sympathetic activation, too [35]. Also components of BPV were lowered (especially LFs-sBP). Discrepancies may be results of different investigated groups and protocol of our investigation in contrary to the Weise, Sanchez-Gonzales and Ghiazi experiments [30, 33, 34].

Previous studies, which evaluated the influence of the acoustic stressor showed responses in ANS activity and the cardiovascular system such as an increase in HR, dBp, LF, and a decrease in the HF parameters of HRV analysis. [36–40] Some parts of these investigations evaluated influences of different kinds of music, which can decrease not only HF, but also LF or even HR [36, 37]. Our observations were different. The one and only component which changed significantly after the acoustic stressor was the HF component of diastolic blood pressure, which is indicative of reduced activation of the parasympathetic nervous system. These differences result from the features of the acoustic stressor used, i.e., its duration, pitch (wave frequency), and sound intensity. Similar changes were observed by Cheng *et al.* [36], but in the HRV, not the BPV analysis. In our study, the HF components of HRV changed differently, the HFnu decreased, but interestingly the HF-RRI increased. Ekuni *et al.* [39] also have somewhat different observations from ours. During their stress procedure, HRV analysis showed a significantly lower level of HF and higher levels of LF and LF/HF. These findings were confirmed in our previous study, Przybylska-Feluś *et al.* [41], in which sympathetic activation in healthy volunteers and celiac patients was detected in response to the sound stressor. There was a significant increase in LFnu and decrease in HFnu parameters of HRV. The response depended on the resting activity of the ANS, and excessive ANS activity caused less response to stimulation. Now, we observed only decreased parasympathetic activation in the dBpV. Walker *et al.* [40] proved that sound stimuli lead to a decline in SDNN. In our study, we observed the same effect, but it was not statistically significant. Walker *et al.* further observed an

increase in both systolic and diastolic blood pressure after exposure to the stress, whereas we observed only an increase in systolic blood pressure, with a decrease in both DBP and mBP. These observations suggest that ANS activation by sound stimuli depends on the specific kinds of stressors employed in the study.

The novelty of our investigation was that we not only evaluated differences in responses to the stressors in the time and frequency domains of HRV analysis, but we also used parameters of nonlinear analysis of HRV. According to Buccelletti *et al.* [42], Approximate Entropy [ApEn] is degree of heart beats' irregularity, with greater values of ApEn correlating to decreased regularity. We noticed a significant increase in this parameter in the acoustic startle group, which could prove that this type of stress increases heart beats' irregularity. According to Carrillo *et al.* [43], elevation of SD1 and SD2 from the Poincare plot is connected with parasympathetic nervous system inhibition. The CPT group has statistically significant lower levels of SD1, which may suggest that this powerful stressor strongly inhibits the parasympathetic system.

Analyzing the response to two different stress stimuli, the CPT and the acoustic startle, showed that in healthy young people whose cardiovascular regulating mechanisms are normal, the acute stimulation of the ANS persists for at least 5 minutes. Thus, the constant inundation of people by multiple similar stress stimuli (such as phone sounds) within a typical 24 hour period can be considered a major "silent aggressor" threatening the long-term cardiovascular health of young and old patients alike.

Conclusions

The Cold Pressor Test has a greater stimulatory effect on the sympathetic autonomic system in comparison to the unexpected acoustic startle stress. Regardless of whether the stimulation originates from the central nervous system (acoustic startle) or the peripheral nervous system (CPT), the final response is demonstrated by an increase in the low frequency components of blood pressure variability and a decrease in the low and high frequency components of heart rate variability.

Acknowledgments

This study was supported by funding from research grant no. K/ZDS/005582 from the Polish Ministry of Science and Higher Education, Warsaw, Poland.

Conflict of interest

None declared.

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