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A computational insight into cardiac beat-to-beat intervals variability: from normal to pathological

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SUMMARY. Over the past 100 years, electrocardiography (ECG) has provided essential information for diagnosing patients with cardiovascular diseases. Despites the large clinical usefulness of classic paper printed 12-lead ECG, along with the accelerated development of computerized data acquisition, beat-to-beat methods of ECG analysis were developed, providing new information in the field of heart rate variability (HRV). The objectives of the study were to establish HRV parameters behavior in healthy subjects and in diabetes mellitus patients, as well as to evaluate HRV during acute cigarette smoking. The results concluded that HRV parameters describe statistically significant lower values in diabetic patients compared to healthy subjects, signifying a decreased response of the heart conduction system to autonomic stimuli. Concerning smoking, the study concluded that during smoking and ten minutes after, HRV parameters presented lower values than before smoking, as revealed by visual (2D and 3D) and analytical HRV methods. Our study is one of the few in literature that focuses on the acute effects of cigarette smoking, rather than the well-known long term effects of this wide-spread habit.

Keywords: computerized instrumentation, electrocardiography, fast Fourier transforms, MATLAB, nonlinear dynamical system.

Introduction

The electrocardiogram (ECG) is a powerful clinical tool in diagnosing heart conditions, providing information on heart rhythm and perfusion of myocardial territories. Since its discovery in 1901 by the Dutch physiologist Willem Einthoven, ECG has been continuously improved and nowadays it is a fundamental tool for screening and diagnosing patients of all ages, in all medical fields, especially cardiology, internal medicine, anesthesiology and intensive care units (Noble *et al.,* 1990; Aubert *et al.,* 2002; Aubert *et al.,* 2003; Qu and Weiss, 2006).

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Traditionally, an ECG examination is characterized by a real time paper recording of a 12-lead signal, which is further evaluated by the physician (Aubert *et al.,* 2002). Recently, computerized ECG measurements gained ground as a consequence of various benefits:

- Lower cost of the ECG device (although a computer is necessary);
- The possibility of unlimited data storage (including the ECG history of the patient, thus eliminating the patient burden of carrying medical documents);
- The possibility of transmitting ECG data by internet to another healthcare specialist (telemedicine);
- The possibility of further analysis of the ECG signal by software methods (Carel, 1982; Hongo and Goldschlager, 2006).

The last category includes heart rate variability (HRV), which is a temporal variation between sequences of consecutive heart beats, as a consequence of the influences of the autonomic nervous system (ANS) on the heart rate (Lokhandwala and Rodriguez, 1999; Acharya *et al.*, 2006). Thus, the fluctuations of heart rate (as analyzed by HRV) are a noninvasive and accurate method of evaluating the balance between the ANS components: the sympathetic component, which prepares the body for the fight-or-flight reaction and translates in an increase of the heart rate, and the parasympathetic component, which prevails when the organism is in a relaxed state, translating in a decrease of the heart rate (Task Force of the European Society of Cardiology, 1996; Acharya *et al*., 2006; Tsai *et al.,* 2014). Certain studies state that there are certain diseases that alter the function of the peripheral nerves, like diabetes mellitus or some neuropathies, resulting in decreased values of HRV parameters (Task Force of the European Society of Cardiology, 1996; Acharya *et al.,* 2006; Orlov *et al.,* 2012; Gardim *et al*., 2014).

HRV is evaluated by three categories of parameters, summarized in Table 1 (Task Force of the European Society of Cardiology, 1996; Aubert *et al.,* 2002; Carvajal *et al*., 2005; Acharya *et al.,* 2006; Billman, 2013).

In relation to interpretation of HRV parameters, most studies admit that decreased HRV values are associated with a loss of heart conduction system sensitivity to autonomic stimuli or with decreased autonomic activity (Task Force of the European Society of Cardiology, 1996; Taelman *et al.,* 2009; Chevalier and Sinatra, 2011; Orlov *et al.,* 2012; Jaiswal *et al.*, 2013; Gardim *et al*., 2014). Also, many authors consider the LF/HF ratio as a measure of sympathetic to parasympathetic balance (von Borell *et al.,* 2007; Taelman *et al.,* 2009), an increase in this ratio being associated with a shift towards sympathetic dominance; however, other authors demonstrate that this hypothesis is false, and state that LF/HF ratio cannot accurately quantify the balance between autonomic nervous system components (Billman, 2013).

The literature is controversial regarding the normal values of HRV parameters. By that reason, most of HRV studies conduct measurements on healthy subjects, in order to establish their own normal values (Nunan *et al.*, 2010).

In addition to the above-mentioned parameters, HRV can be promptly analyzed by visual methods, such as the tachogram, the histogram and the Poincaré plot of beat-to-beat intervals (Task Force of the European Society of Cardiology, 1996; Mirescu and Harden, 2012a; Makivic *et al.*, 2013).

Table 1.

HRV parameters

Parameter	**Unit**	**Significance**
Time-domain parameters		
Mean RR	ms	Mean of consecutive beats intervals
STD RR	ms	Standard deviation of consecutive beats intervals
Mean HR	beats/min	Heart rate mean
SD HR	beats/min	Standard deviation of heart rate mean
NN50	#	Number of consecutive heartbeats with a difference of at least 50 ms
pNN50	%	Percent of consecutive heartbeats with a difference of at least 50 ms
Triangular index	-	The integral of the density distribution divided by the maximum of the density distribution
TINN	ms	The baseline with of the intervals histogram
Frequency-domain parameters (obtained by fast Fourier transforms)		
LF/HF	-	Ratio between low frequency spectral power and high frequency spectral power
Nonlinear dynamics parameters		
SD1	ms	Standard deviations according to the Poincaré plot of the intervals between heartbeats
SD2	ms	
ApEn	-	Approximate entropy of the stationary signal
SampEn	-	The likelihood that runs of patterns that are close to each other will remain close in the next incremental comparisons
Correlation dimension	-	Entropy parameters
DFA α1	-	
DFA α2	-	

Materials and methods

Essentially, any method that records a heartbeat-derived signal is appropriate for HRV parameters evaluation. There are two fundamental methods used for HRV: electrocardiography and photopletismography (PPG). Both portray cardiac activity indirectly: the ECG representing the cyclic electrical signal evoked by the cardiac cells, and the PPG expressing peripheral blood volume variation, which is dependent of the cardiac output (Figure 1).

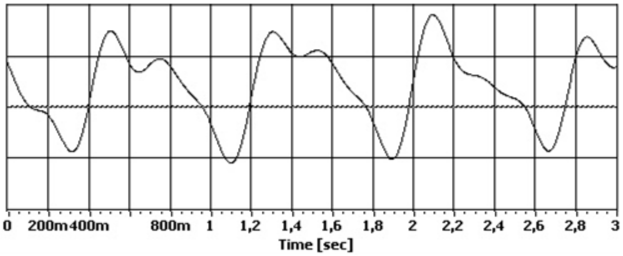

Figure 1. Example of a PPG signal

Each of these signals is characterized by cyclic waves that can be identified by software methods, and further used to calculate beat-to-beat intervals that are used for HRV studies. The cyclic waves correspond with systole (the period where the heart contracts and expels the arterial blood to the arteries) and diastole (the period where the heart is relaxed and receives venous blood from the veins).

A PPG-based recording device is an optical system composed of an infrared light emitting diode (LED) and a photodiode, which are placed on the sides of a finger (or earlobe). Due to the fact that oxygenated blood absorbs infrared light and blood flow is pulsatile as a result of the intermittent cardiac pump function, the result is a pulsatile wave (Figure 2) (Mirescu, 2015).

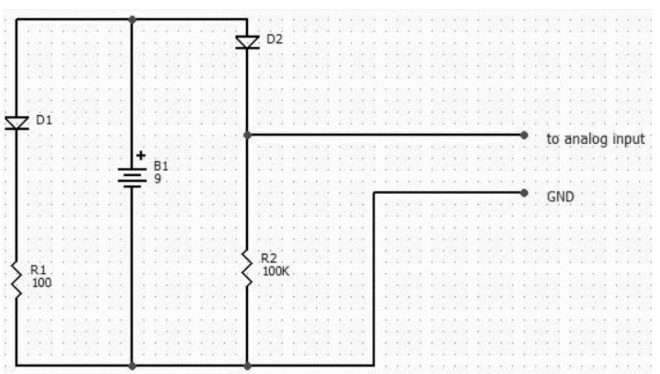

Figure 2. Schematics of a simple PPG device designed for HRV purposes (Mirescu, 2015 – reproduced with permission)

Albeit PPG is a simple and convenient method for HRV purposes, and its results are equivalent to the ones obtained from ECG traces, there are a series of disadvantages in using this procedure, the most important being an increased sensibility to movement artifacts (Mirescu *et al.*, 2016).

Although there is a functional equivalence between the two methods (Mirescu and Harden, 2012b), ECG proved to be the much more reliable in recording and tracing heart beat signal for HRV. Figure 3 shows the block diagram of a classical digital ECG device.

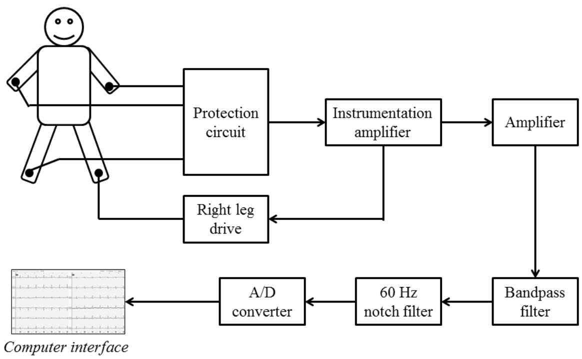

Figure 3. Block diagram of a classical digital ECG device

At the base of ECG recordings is the so called „triangle of Einthoven" (Figure 4), describing in a graphical manner the main ECG leads, which basically represent the potential difference between three key points located on the body surface: the right arm, the left arm and the left leg.

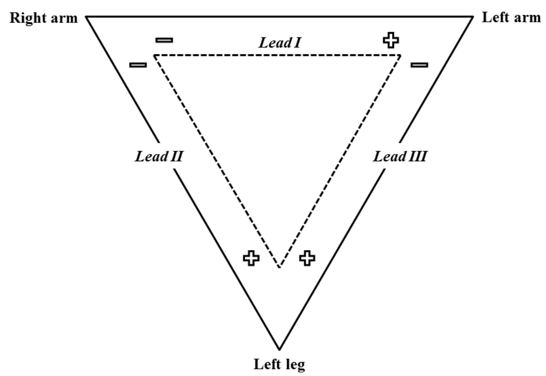

Figure 4. The triangle of Einthoven

Experimental design

The aims of the study were (1) to establish normal values of HRV variability parameters on a Romanian sample population of healthy individuals; (2) to examine HRV parameters values and visual descriptors of diabetic patients; (3) to evaluate the influence of acute cigarette smoking on HRV parameters.

In order to establish normal values for HRV parameters, 50 healthy subjects were taken into consideration (age 22-57 years old; 23 females). For each subject, we recorded a 10 minutes ECG trace using a Neurosoft Poly-Spectrum® device, in a 6-lead configuration (standard ECG leads DI, DII, DIII, aVR, aVL, aVF). The device software automatically identified the R waves of the ECG trace and calculated the intervals between consecutive beats, in milliseconds (RR intervals), as shown in Figure 5.

Figure 5. Three consecutive RR intervals as measured by Poly-Spectrum® software, in aVF lead (N symbolizes that the corresponding beat is normal, not ectopic)

The subjects were asked not to smoke or drink caffeinated beverages or other psychostimulants for four hours prior to the recordings. They were also asked not to speak or move during the recording, in order to avoid artifacts.

For evaluating the effects of diabetes mellitus on HRV parameters, we selected 25 diabetic patients admitted to the Diabetes, Nutrition and Metabolic Diseases Center, Emergency County Hospital Cluj, (22-70 years old, 12 females). Each patient was subjected to a 10 minutes ECG recording, using the same device and experimental setting as described before. Medical data was anonymously collected from the patients' medical records. In collecting data from the patients, ethical standard was considered, and patients were not required to discontinue any prescribed treatment and care was taken for the patients not to suffer any discomfort during the recordings.

For evaluating the acute effects of cigarette smoking in healthy subjects, a number of 25 volunteers were recruited (age 20-22 years, 15 females). The recordings were made according to the following protocol:

- The subjects were asked not to smoke or consume psychostimulants at least 4 hours prior to the experiment;
- 6-lead ECG was recorded in a resting position for 5 minutes (baseline recording);
- 5 minutes – ECG recording while smoking a cigarette;
- 10 minutes recording after smoking. This interval was divided into two 5 minutes intervals, and for each HRV parameters were calculated.

The ECG measurements were performed using the same device as described before.

In all three experimental settings, HRV parameters were calculated using *Kubios HRV 2.2* software. Statistical analysis was performed using *Microsoft Office Excel 2010*, and 3D plotting was accomplished using the *scatter3* function of the MATLAB environment.

Data collected from diabetic patients were compared to data obtained from the healthy subjects.

Parameters obtained from the ECG recordings during (and after) cigarette smoking were compared to the ones obtained from the baseline recordings of the same subjects.

Results and discussion

A. Normal values of HRV parameters

HRV parameters values obtained from healthy subjects and considered normal in our studies are illustrated in Table 2.

Table 2.

Normal values of HRV parameters

Parameter	**Unit**	**Value**		
Time-domain parameters				
Mean RR	ms	745.024	±	100.426
STD RR	ms	51.69673	±	18.80544
Mean HR	beats/min	82.32475	±	9.924824
SD HR	beats/min	5.66064	±	34.49615
NN50	#	70.58333	±	86.61208
pNN50	%	14.04793	±	16.26272
Triangular index	-	12.72905	±	4.72086
TINN	ms	246.875	±	89.3929
Frequency-domain parameters				
LF/HF	-	3.191386	±	2.383248
Nonlinear dynamics parameters				
SD1	ms	27.77991	±	15.11479
SD2	ms	68.34148	±	24.21562
ApEn	-	1.16733	±	0.146363
SampEn	-	1.392332	±	0.264679
Correlation dimension	-	2.63317	±	1.3481
DFA $\alpha1$	-	1.213387	±	0.279406
DFA $\alpha2$	-	0.859523	±	0.160867

The data obtained in our study are consistent with the literature data gained from other populations of matching sex and age (Aubert *et al.*, 2003; Nunan *et al.*, 2010). Standard deviation of some parameters (NN50, pNN50) was higher than the average of the parameter value, signifying a broad range of values, hence an important variability among healthy subjects.

B. HRV parameters in diabetic patients

We compared all three categories of HRV parameters (time-domain, frequency-domain and nonlinear dynamics parameters) for healthy subjects and diabetic patients. T test was used to compare the parameters between the two groups (p values lower than 0.05 were considered statistically significant). The results are presented in Table 3.

Table 3.

HRV parameters in diabetic patients and the p-value of the t test compared to healthy subjects

Parameter	Unit	Value	p-value
Time-domain parameters			
Mean RR	ms	762.80 ± 100.77	0.57
STD RR	ms	25.96 ± 14.58	< 0.001
Mean HR	beats/min	79.79 ± 13.3	0.45
SD HR	beats/min	2.56 ± 1.12	< 0.001
NN50	#	19.76 ± 37.69	0.04
pNN50	%	2.47 ± 4.85	0.01
Triangular index	-	7.01 ± 3.73	< 0.001
TINN	ms	145 ± 88.6	< 0.001
Frequency-domain parameters			
LF/HF	-	3.03 ± 1.96	0.8
Nonlinear dynamics parameters			
SD1	ms	11.56 ± 9.56	0.004
SD2	ms	34.3 ± 19.25	< 0.001
ApEn	-	1.23 ± 0.16	0.16
SampEn	-	1.13 ± 0.66	0.72
Correlation dimension	-	0.69 ± 0.6	< 0.001
DFA α1	-	1.19 ± 0.26	0.8
DFA α2	-	1.06 ± 0.23	< 0.001

According to our results, all HRV parameters of diabetic patients expressed decreased values, compared to healthy subjects. These results are consistent with other studies in the literature performed on diabetic subjects (Task Force of the European Society of Cardiology, 1996; Orlov *et al.*, 2012; Gardim *et al.*, 2014). SDNN, a marker of overall HRV, was 25.96 ms in diabetic patients, compared to 51.69 in healthy subjects ($p < 0.001$), which is in accordance with other studies (Jaiswal *et al.*, 2013).

Another finding of our study was decreased values of NN50 and pNN50 in diabetic subjects, compared to the control group ($p = 0.04$ and $p = 0.01$, respectively). Many of the diabetic patients had the value 0 at these parameters, a situation not described so far in the literature, implying that a decreased value of these parameters could be an independent descriptor of HRV in DM patients. However, further research needs to be conducted in order to strengthen this conclusion.

A powerful visual descriptor of HRV is the tachogram of the RR intervals, which represents the plotting of the RR intervals against time. In a typical 10 minutes recording of a healthy person, numerous inflections and deflections can be seen, due to the antagonistic and continuous influence of the two components of the autonomous nervous system: the sympathetic nervous system creates a downwards inflection (signifying an increase in heart rate) and the parasympathetic nervous system creates an upwards inflection (signifying a decrease in heart rate). Another cause of these irregular changes in the heart rate is the so-called respiratory sinus arrhythmia, a physiological phenomenon which consists of heart rate increasing during inhaling and decreasing during exhaling (von Borell *et al*., 2007; Tininenko *et al.*, 2011; Mirescu and Harden, 2012a,b; Mirescu, 2015). As seen in Figure 6, the tachogram of diabetic patients describes a visible decrease in variability compared to a healthy subject, the tachogram being almost flat. As it is also shown in other studies (Orlov *et al.*, 2012; Jaiswal *et al.*, 2013; Gardim *et al*., 2014), the almost flat appearance of the RR intervals tachogram is an independent marker of overall decreased HRV in diabetic patients and is correlated with the severity of the disease.

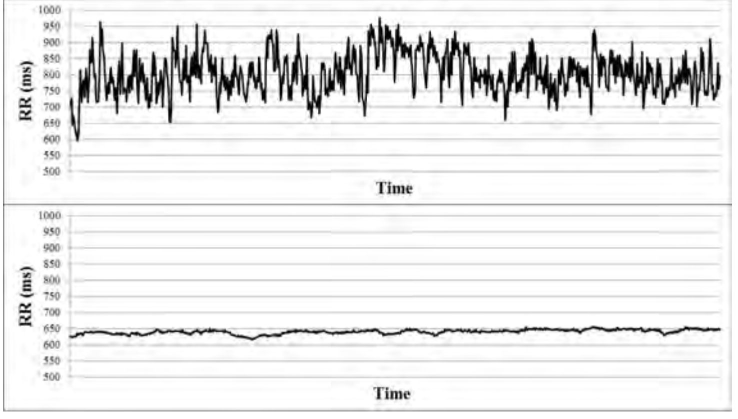

Figure 6. Up – Tachogram of RR intervals in a healthy subject; down – tachogram of a diabetic patient, which displays a decrease in the variability of the intervals; both recordings were made on the standard 10 minutes interval.

Another valuable visual descriptor of HRV is the Poincaré diagram (scattergram), which is a scatter plot of every RR interval against the previous one. As many authors state, the Poincaré diagram is a graphical projection of RR_{i+1} as a function of RR_i (Task Force of the European Society of Cardiology, 1996; Medeiros, 2010; Makivic, 2013). It reflects the graphical correlation between consecutive RR intervals and has become increasingly more popular due to its simple visual interpretation and its proven clinical applicability (Brennan et al., 2001). The Poincaré plot from a healthy subject expresses a large surface of the scattered points, without many isolated points from the main cloud (which may represent ectopic beats or artifacts). As seen in Figure 7, the points of the Poincaré plot of a diabetic patient are significantly aggregated into a much smaller cloud than the ones of a healthy control subject. These visual findings are in conformity with the mathematical descriptors of the Poincaré plot (SD1 and SD2) for diabetic patients.

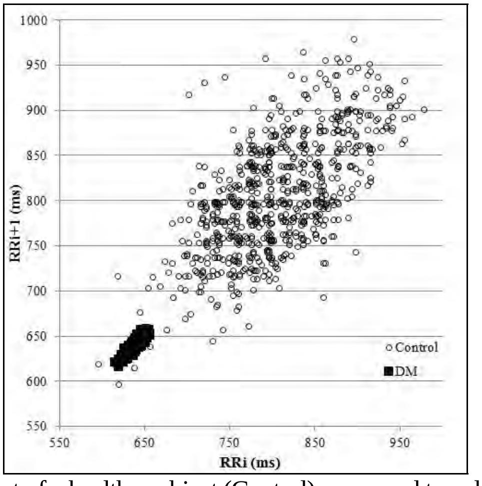

Figure 7. A Poincaré plot of a healthy subject (Control) compared to a diabetic patient (DM)

In an attempt to enhance the information provided by this method, we tested the value of 3D Poincaré plots (RRi+2 and RRi+1 as a function of RRi). The 3D plotting was performed in MATLAB environment, using the *scatter3* function, for all diabetic subjects. As shown in Figure 8, apart from a better visual representation, the 3D scattergram does not provide further information for HRV interpretation purposes.

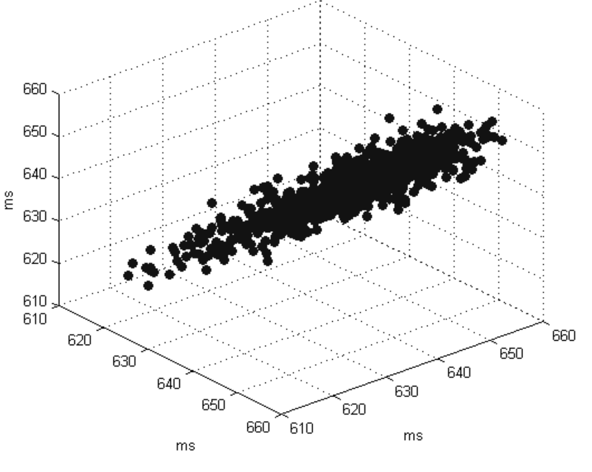

Figure 8. Up – A 3D Poincaré plot of a diabetic patient

As previous studies state, the decrease of HRV in diabetic patients is the result of nerve fiber alteration.

Our study concludes that HRV analysis (by visual and computational methods) is a valuable instrument in autonomic (both sympathetic and parasympathetic) response in diabetic patients, especially in patients which present other autonomic dysfunctions (e.g. altered bowel movement, decreased sweat response or skin ulcers). Most of the cited studies evaluate HRV response in diabetic children and young patients (with type I diabetes mellitus), but the results are consistent with the ones obtained in our study.

C. Influence of acute cigarette smoking on HRV parameters

Although the effects of chronic consumption of tobacco are well known, few studies explored the immediate consequences of cigarette smoking on the functioning of the cardiovascular system. We characterized these effects by the visual and computational methods of HRV.

Time-domain (Mean RR, STD RR, Mean HR, STD HR, pNN50), frequency-domain (LF/HF) and nonlinear dynamics (SD1, SD2) parameters were calculated for each measure interval described in the protocol (5 minutes before smoking, five minutes during smoking and two consecutive 5 minutes interval after smoking). T test was used to compare amongst recordings (p values lower than 0.05 were considered statistically significant).

As expected, the average of RR intervals increased during smoking ($p < 0.001$), decreasing in the next 10 minutes ($p = 0.03$, $p = 0.05$ respectively) – Figure 9A.

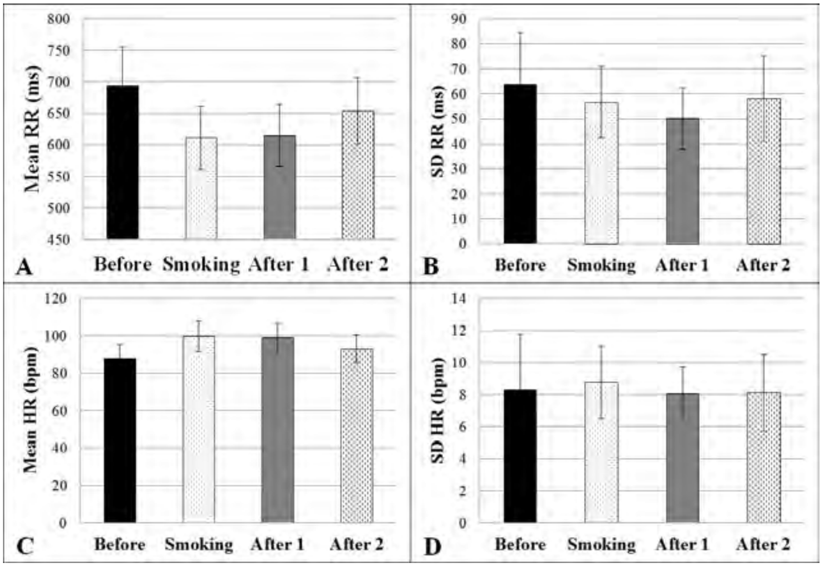

Figure 9. Time-domain parameters (A – Mean of RR intervals; B – Standard deviation of RR intervals; C – Mean heart rate; D – Standard deviation of momentarily heart rates).

The standard deviation of RR intervals (a global measure of HRV) decreased during smoking and continued to decrease 5 minutes after smoking, following an upward trend in the last 5 minutes of the recording, but none of these differences were statistically significant compared to the control recording (Figure 9B). Average heart rate and the standard deviation of the momentarily heart rates followed the same pattern (Figure 9C, D). pNN50, another global descriptor of HRV, had the same evolution as the standard deviation of RR intervals, but, as stated in other studies study, the values were dispersed, and the standard deviation was very high, making this parameter inaccurate for interpretation (Figure 10A).

Figure 10B shows the variation of LF/HF (ratio of the frequency-domain parameters) during the four recording periods. Although there were no significant differences, LF/HF increased during smoking and in the 5 minutes period after smoking, being in accordance with the alteration of other sympathetic prevalence parameters (the decrease of STD RR and pNN50).

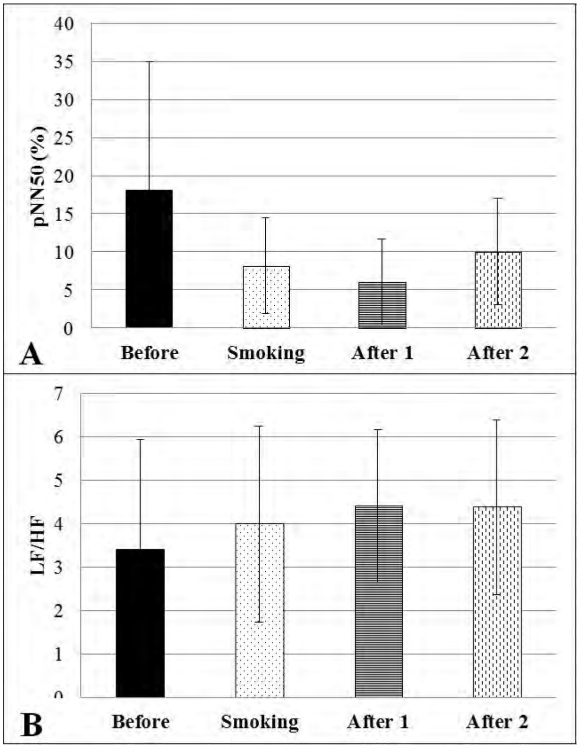

Figure 10. A– The independent descriptor of HRV, **pNN50** decrease during smoking. The decrease continued in the first interval after smoking, but the parameter showed a tendency to return to baseline in the second interval; B – LF/HF ratio increased during smoking, similar to the sympathetic activity increase.

The Poincaré plot derived from the four recording periods is the most valuable visual instrument in evaluating HRV alterations. During smoking, the increasing in sympathetic tonus is represented by the clustering of the points in smaller cloud than in the control cloud (Figure 11), which is also sustained by the mathematical parameters of the Poincaré plot (SD1 and SD2 are lower during the smoking period, $p > 0.05$). During the following two periods, the dispersion of the points (thus, the area of the scattergram) began to increase, but, even after 10 minutes from the cessation of smoking, it had not reached the initial appearance.

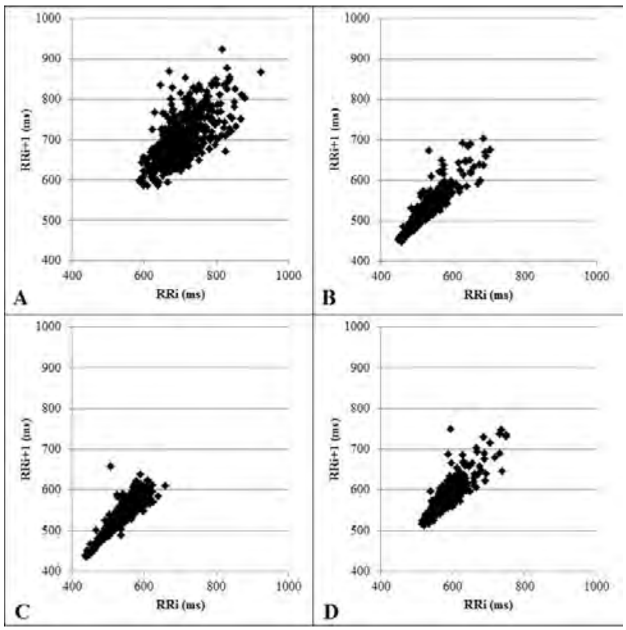

Figure 11. Poincaré plot of a subject derived from HRV recordings during the four periods (A – Before smoking, the Poincaré plot has a large dispersion of points, which shows a heart rate variability increase; B – During smoking the dispersion of points decreased and they move to the left-down, suggestive for a increase sympathetic activity ; C – 5 minutes after smoking the effect is similarly to B period; D – 10 minutes after smoking the points begin to deviate from the origin, which means an increase in variability and a decrease in the sympathetic activity).

Our study regarding the acute effects of smoking on heart rate and HRV is one of the few in literature and one of the most complex, considering that it considered many HRV parameters and visual indicators of HRV.

Other authors obtained similar results in a smaller scale study, concluding that smoking has a global decreasing effect on HRV parameters and that after some time they return to the values before smoking (Gondim *et al.*, 2015). On the contrary, a larger scale study concluded that smoking does not have any influence on HRV parameters in male adolescents, nor does passive smoking, thus the habit does not have any effects on autonomic modulation changes (Karakaya, 2007). Also, the above-mentioned study concluded that there were no differences in HRV parameters between smokers and non-smokers. These differences could come from not taking into consideration all smoking habits of the subjects (number of cigarettes smoked, concentration of nicotine, and personal history of smoking in years).

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