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# Nonlinear properties of cardiac rhythm and respiratory signal under paced breathing in young and middle-aged healthy subjects

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ABSTRACT

We examined the effects of gender and age in young and middle-aged subjects on the level of cardio-respiratory interaction by analyzing properties of cardiac, respiratory and cardiac-respiratory regulatory mechanisms under paced breathing. In 56 healthy subjects, ECG (RR interval) and respiratory signal were simultaneously acquired in supine position at paced (0.1–0.45 Hz, steps of 0.05 Hz) and spontaneous breathing. The participants were divided into gender matched group of young adults (19–25 years old) and middle-aged adults (35–44 years old). Power spectral analysis was applied on RR interval time series and spectral components in very low frequency (VLF), low frequency (LF) and high frequency (HF) ranges were computed. We also calculated sample entropy of RR interval series (SampEnRR), respiratory series (SampEnResp), and their cross-sample entropy (cross-SampEn). Under paced breathing, reduction of all spectral powers with age ($p < 0.05$) is not gender dependent but reduction of some entropy measures is; SampEnRR and SampEnResp were lower only in men ($p < 0.05$). In the middle-aged subjects, effect of gender on spectral measures is significant; males had lower HF ($p < 0.05$). Pattern of dependencies of SampEn and cross-SampEn on paced breathing frequency were significantly different in men (young *vs.* middle-aged, $p = 0.001$ and $p = 0.037$) and in middle-aged subjects (females *vs.* males, $p = 0.011$ and $p = 0.008$). In middle-aged males, lower entropy measures indicated reduced and less complex partial cardiac and respiratory control, and central cardio-respiratory control. In conclusion, in healthy middle-aged subjects changes in cardio-respiratory coupling are detectable only in males.



## 1. Introduction

In recent times, a great progress has been made in the study of properties of complex physiological systems and underlying regulatory mechanisms. The new findings are coming from application of newly and sophisticated classical time series analyzing methods on one or more simultaneously recorded physiological signals. Electrocardiogram (ECG), particularly, interbeat (RR) interval or heart rate (HR) signal is one of the most explored physiological time series. Spectral analysis of heart rate variability (HRV) was introduced as a noninvasive technique in quantification of cardiovascular control by autonomic nervous system (ANS) activity [1,2]. It is accepted that high frequency spectral component (HF) is a measure of cardiac vagal modulation [2] while low frequency (LF) spectral component is still the most controversial parameter in literature. Its ability to quantify cardiac sympathetic modulation was questioned in several papers [3–6]. Very low frequency (VLF) spectral component quantifies several mechanisms; in addition to rennin–angiotensin system, it is also influenced by thermoregulation [7], neuroendocrine oscillations [8], chemoreflex related [9] and vagal control of the heart [1,5]. The cardiac and respiratory systems are dominantly neurologically and mechanically coupled and different aspects of cardio-respiratory interaction were found by quantification of the various types of the association between them [10–12]. The respiratory influence on heart rhythm was also quantified by spectral analysis of HRV in experiments with paced breathing. It was shown that increase in breathing frequency (BF) was followed by reduction of total HRV [13,14] while paced breathing at 0.25 Hz didn't affect spectral measures *i.e.* ANS activity [15].

Decline of linear HRV parameters with age appeared steeper in the younger than in the older subjects [16] and together with nonlinear HRV data indicated on a reduction of autonomic cardiac control [5,17,18]. However, data about gender based differences on

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HRV indices in the aging process are controversial and suggest that gender differences vanish after 40 years [18] or later [17,19], but mechanisms in gender-dependent ageing on the level of cardio-respiratory interaction are unknown.

Besides linear HRV parameters, which describe variance of beat-to-beat intervals, nonlinear parameters have been developed to quantify regularity/complexity of time series, which resulted in higher sensitivity for detecting disturbances in underlying physiological regulatory mechanisms. The most applied were the methods that quantify scaling properties of HRV, scaling exponents and fractal dimension [20,21] and the methods based on entropy (approximate or sample entropy) as a complexity measure of HRV [22,23]. Richman and Moorman [24] introduced sample entropy as corrected apparent entropy method invented by Pincus [22] as a measure of irregularity/complexity in time series. Higuchi fractal dimension [25] is also a measure of signal complexity. Qualitatively speaking, it is calculated as the rate of decline of signal length while the number of samples is being reduced. It should be expected that length of more complex signals, usually having higher frequency spectral components, decreases more swiftly than the simpler ones. Recently data have shown that methods based on quantification of scaling properties as scaling exponents and Higuchi fractal dimension are strongly related to spectral components [5,26,27] and only methods based on entropy calculation are still complementary with power spectrum analysis.

It is known that various health issues as metabolic or neurocardiological disorders are associated with the changes in the function of cardiovascular and respiratory system. However, the physiological background of cardio-respiratory coupling and age and sex related impacts still are not sufficiently clarified and it has yet to be investigated. Complementary analysis methods and physiological perturbations are usually powerful tools in revealing hidden mechanisms of well-known physiological phenomena. Hence, we aimed to examine effects of age and gender on complexity and variability of cardiac rhythm and respiratory signal under paced breathing with attempt to elucidate gender based difference at the beginning of aging process in healthy subjects.

## 2. Methods

### 2.1. Subjects

Fifty six healthy subjects (age range, 19 to 44 years) were included in this study. The subjects were divided into 2 groups: (1) 26 young adults, 19 to 25 years old (mean, $22.50 \pm 0.61$ years) with body mass index BMI (mean, $22.6 \pm 1.0\,kg/m^2$) and (2) 30 middle-aged, 35 to 44 years old (mean, $40.27 \pm 0.51$ years) with BMI (mean, $24.70 \pm 0.70\,kg/m^2$). In each group, the number of men and women was equal. The subjects were apparently healthy, with no history or symptoms of heart disease, hypertension, or diabetes, and with normal findings on clinical examination. The study was approved by the Ethics Committee of Faculty of Medicine University of Belgrade, and each subject signed an informed consent.

### 2.2. Experimental protocol

The subjects were asked to avoid physical activity, food, alcohol, coffee and tea 2 h before the experiment. The experiment was performed at 13.00 p.m. in quiet laboratory conditions, in the supine position, at the Institute of Biophysics, Faculty of Medicine in Belgrade. The procedure involved the following design: 20 min of relaxation with spontaneous breathing frequency and eight sessions with paced breathing tasks in the frequency range (0.1–0.45) Hz, with step 0.05 Hz. Each task lasted 10 min and they were separated by pauses of 5 min. The stimuli by which participants paced their breathing were generated from a computer web metronome (webmetronome.com). The subjects were instructed to inhale and exhale with two different auditory tones, equidistant in time. In almost all cases, participants breathed almost exactly at the "target breathing rate". All subjects performed tasks succesfully. No restriction was imposed on tidal volume, although subjects were instructed to mantain a comfortable level of ventilation.

### 2.3. Data acquisition

The ECG and respiratory data were simultaneously acquired with sampling frequency of 1000 Hz by a Biopac MP100 system with AcqKnowledge 3.9.1 software (BIOPAC System, Inc, Santa Barbara, CA, USA). The ECG data were collected using the ECG 100 C electrocardiogram amplifier module for the measurement in R-mode (R wave only). The classic limb lead I based on 3 electrodes attached to the hand wrists and leg was used. The RSP 100 C respiratory pneumogram amplifier module with TSD 201 transducer attached to the belt (adjustable nylon strap) was used to measure abdominal expansion and contraction. Transducer was placed on the abdomen, at the point of minimum circumference (maximum expiration).

RR(t) intervals were determined by Origin 6.0 (Microcal Software, Inc., Northampton, MA, USA) software. Individual spontaneous breathing frequencies were determined as reciprocal values of mean value of IBI—inter breath interval series, also determined by Origin 6.0.

Respiratory signal was low pass filtered (4th order Chebyshev filter) with the cutoff frequency individually customed and set to a value less than 2 Hz, typically around 1 Hz. This allowed us to eliminate small amplitude jitters that existed in the signal. Since respiratory signal Respf(t) was uniformly sampled with sampling frequency of 1 kHz, while samples of RR(t) were unequally positioned, equal equidistant resampling of Respf(t) and RR(t) was done with our original MATLAB program using mean value of RR(t) for each individual and each task data. The resampling procedure was performed by linear interpolation between two corresponding adjacent existing samples.

### 2.4. Power spectral analysis

The fast Fourier transform (FFT) with Hanning window function was used to calculate power spectrum density from the series of 512 RR intervals (Fig. 1). The spectral components were determined carrying out integration of the power spectrum in spectral bands, as follows: very low frequency (VLF) in the range 0.004–0.04 Hz, low frequency (LF) in the range 0.04–0.15 Hz, and high frequency (HF) in the range 0.15–0.50 Hz and total power (TP) in the range 0.004–0.5 Hz [5]. To capture the whole effect at paced breathing frequency at 0.1 Hz, exception was made, and low frequency spectral power was calculated in the range 0.04–0.16 Hz and HF in the range 0.16–0.5 Hz. Average value of spectral components from two 512 data segments was used as rest data.

#### 2.4.1. Sample entropy and cross sample entropy

Sample entropy (SampEn) is a measure of irregularity (unpredictability) in time series data [24]. It is defined as the negative natural logarithm of the conditional probability that two sequences similar for $m$ points remain similar within tolerance $d$ at the next point, where self-matches are not included. We calculated SampEn from RR(t) interval series and corresponding respiratory signal Respfr(t) with fixed input variables $m = 2$ and $d = 0.2$ SD, SD being the standard deviation of series. Small SampEn values are associated with pattern regularity within a signal, and larger SampEn values are associated with greater complexity. Cross sample entropy (cross-SampEn) as a nonlinear measure of coupling between two