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## **Heart Rate Nonlinear Dynamics during Sudden Hypoxia at 8,230 m Simulated Altitude**

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### **Running title**

Heart rate dynamics during hypoxia

## Abstract

*Background:* Acute hypobaric hypoxia is associated with autonomic changes that bring a global reduction of linear heart rate variability (HRV). Although changes in nonlinear HRV can be associated with physiological stress and are relevant predictors of fatal arrhythmias in ischemic heart disease, to what extent these components vary in sudden hypobaric hypoxia is not known. *Methods:* Twelve military pilots were supplemented with increasing concentrations of oxygen during decompression to 8,230 m in a hypobaric chamber. Linear and nonlinear HRV was evaluated at 8,230 m altitude before, during and after oxygen flow deprivation. Linear HRV was assessed through traditional time- and frequency- domain analysis. Nonlinear HRV was quantified through the short term fractal correlation exponent  $\alpha_s$  and the Sample Entropy index (SampEn). *Results:* Hypoxia was related to a decrease in linear heart rate variability indexes at all frequency levels. A non significant decrease in  $\alpha_s$  (basal:  $1.39 \pm 0.07$ , hypoxia:  $1.11 \pm 0.13$ , recovery:  $1.41 \pm 0.05$ ,  $p = 0.054$ ) and a significant increase in SampEn (basal:  $1.07 \pm 0.11$ , hypoxia:  $1.45 \pm 0.12$ , recovery:  $1.43 \pm 0.09$ ,  $p = 0.018$ ) were detected. *Conclusions:* The observed pattern of diminished linear heart rate variability and increased nonlinear heart rate variability is similar to that seen in subjects undergoing heavy exercise or in patients with ischemic heart disease and high risk for ventricular fibrillation.

## Key words

Autonomic nervous system, heart rate variability, nonlinear, hypoxia, high altitude

## Introduction

Autonomic responses are crucial for adaptation to hypoxia. Acute hypoxia triggers several autonomic mechanisms, mainly in the respiratory system (e.g., the hyperventilation started by the peripheral chemoreceptor reflex) and in the cardiovascular system (e.g., sympathetic activation with heart rate and cardiac output increase to improve pulmonary and tissue perfusion)<sup>1</sup>. Previous studies have evaluated heart autonomic response using Heart Rate Variability (HRV) analysis in subjects exposed to acute hypobaric hypoxia in real<sup>2</sup> as well as simulated settings<sup>3,4</sup>, usually introducing the changes in inspired O<sub>2</sub> concentration gradually and at altitudes below 6,400 m (21,000 ft).

HRV analysis is based on the fact that the autonomic control of the sinus rhythm adds variability to the heart rate signal at different frequency levels. Among these, in short-term studies (less than 10 min), the high-frequency (HF) component (0.15 - 0.4 Hz) is related to respiratory sinus arrhythmia mediated by parasympathetic activity, and the low-frequency (LF) component (0.04 - 0.15 Hz) is related to the baroreflex control including sympathetic and parasympathetic influences. In this context, the very low-frequency (VLF) component (< 0.04 Hz) is a yet undefined measure which may reflect changes in the trend of data<sup>5</sup>.

In addition to the cyclic fluctuations previously described, there are complex fluctuations of the sinus rhythm that can be analyzed with nonlinear dynamics methods. It is believed that nonlinear fluctuations are determined by interactions of electrophysiological, haemodynamic, and humoral variables, as well as by autonomic and central nervous regulation<sup>5,6</sup>. Changes of nonlinear HRV are associated with several cardiovascular stressors. The decreased nonlinear HRV has been related to physical stress<sup>7</sup>, aging<sup>8</sup>, and a variety of pathological conditions such as stable coronary artery disease<sup>9</sup>. On the other hand, increased nonlinear HRV was also associated with physiological situations such as high exercise intensity levels<sup>10</sup> and complicated ischemic heart disease where it has been shown to be predictive for ventricular fibrillation and sudden cardiac death<sup>11</sup>.

Most of the studies that assessed the relation between HRV and acute hypobaric hypoxia, reported a reduced linear HRV pattern consistent with an increased sympathetic tone and decreased parasympathetic tone<sup>2-4</sup>. Nonlinear indicators of HRV have been sparsely studied in these situations. The results indicate that nonlinear heart rate fluctuations also decrease in acute hypobaric hypoxia<sup>12,13</sup>.

Altitude-induced changes in cardiac rhythm could explain the significant rate of sudden cardiac death at high altitudes<sup>14</sup>. It has been reported that 30% of all deaths during mountain sports at altitude are attributable to sudden cardiac death, more likely in those who have had prior myocardial infarction, coronary artery disease or coronary risk factors<sup>15</sup>. In addition, it has been seen that the occurrence of ventricular extrasystoles is proportional to the altitude during acute hypoxia exposure in healthy elderly man<sup>16</sup>.

However, contrary to what would be expected, the HRV pattern of diminished linear and nonlinear HRV observed when subjects are exposed to altitudes lower than 6,400 m (21,000 ft) does not resemble that of patients with increased risk of death due to cardiac arrhythmias, who display reduced linear HRV but increased nonlinear HRV<sup>11</sup>. It is possible that this discrepancy is related to an insufficient hypobaric-hypoxic stress; therefore, we hypothesized that exposure to an altitude higher than 6,400 m (21,000 ft) may result in an alteration of the HRV pattern more similar to that seen in patients with high risk of arrhythmia-related death.

In the present study we examine the nonlinear heart rate variability pattern associated with severe acute hypoxia aiming to obtain information that could help to understand the autonomic nervous system mechanisms underlying situations related with increased risk of cardiac arrhythmias. In this regard, the use of training protocols followed by aircrews to learn recognition of their symptoms of hypoxia at simulated altitudes of 8,230 m (27,000 ft) in hypobaric chambers<sup>17</sup>, allows the possibility to compare the autonomic responses with those already studied at lower altitudes<sup>12, 13</sup>.

## Methods

### *Ethical Approval*

The study conformed to the standards set by the Declaration of Helsinki, and it was approved in advance by the institutional committee for ethical review of the Argentine National Aerospace Medicine Institute, Buenos Aires, Argentina. All participants provided voluntary written informed consent before taking part. The data were collected non-invasively during routine training that the subjects had to undertake.

### *Subjects*

We studied 17 healthy military male pilots who underwent a sudden hypoxic exposure as part of their standard training procedure. All subjects had been previously subjected to a full medical and psychological examination.

### *Hypoxic protocol*

The study was performed in a hyperbaric chamber (The Biggs Boiler Works Co., Akron, Ohio, USA) adapted for use as hypobaric chamber (Pittsburgh-Des Moines Steel Co., Des Moines, Iowa, USA) at installations of the Argentine National Aerospace Medicine Institute. Participating crews entered into the chamber in groups of six together with a physician. Pressure was lowered to simulate an ascent to 8,230 m (27,000 feet) at a rate of 640 m/min (2,100 feet/min). During the ascent subjects received, through a pressure type mask, a mixture of oxygen and ambient air provided on demand by an oxygen regulator USAF Type CRU-68/A (Aro Corporation, Bryan, Ohio, USA).

The ratio of oxygen to air was automatically adjusted to supply increasing oxygen as altitude increased. At 8,230 m (27,000 feet) all participants were receiving 100% oxygen. Then, oxygen flow was abruptly interrupted for one participant at a time. In order to detect psychomotor impairment each participant had to write on a paper sheet numbers in a descending order starting from 999. As soon as the first symptom of hypoxia appeared the oxygen flow was restarted and the pilot resumed the writing. After the subject was fully recovered and reached basal oxygen saturation levels, the next pilot was evaluated. Oxygen saturation was measured continuously during the test by using a Healthdyne 930 pulse oximeter (Healthdyne Technologies Inc., Marietta, Georgia, USA). After the test was run for all participants, the normal atmospheric pressure was gradually restored.

HRV was analyzed before (basal), during (hypoxia) and after (recovery) oxygen flow interruption. The beats corresponding to the "basal" stage were taken from the period immediately prior to the interruption of oxygen. The transition beats corresponding to the time between the interruption of the oxygen flow and the stabilization of the heart rate at an increased level (as determined by visual inspection) were not considered for the analysis. The beats corresponding to the "hypoxia" stage were taken from the elapsed time after the ending of the transition period and the restoration of oxygen flow. The transition beats corresponding to the elapsed time between the restoration of the oxygen flow and the stabilization of heart rate at basal levels (as determined by visual inspection) were not considered. Finally, the beats corresponding to the "recovery" stage were taken from the period immediately subsequent to this last transition period (Figure 1).

Within each subject, the number of beats selected for the "basal" and "recovery" epoch was the same as the number of beats selected for the "hypoxia" epoch. We selected the same number of beats for all stages because in short time series of less than 200 beats the error associated to nonlinear HRV analysis may vary as a function of data length<sup>6, 18</sup>. As the transition periods between stages were not evaluated, the records were free of pronounced upward or downward trends<sup>5</sup>.

### *Signal recording and HRV analysis*

*Signal recording:* Electrocardiogram signal was recorded using a digital Holter device (Holter HCAA 348 / Holtech / Servicios Computados S.A. / Buenos Aires / Argentina) and stored in a solid-state memory. Ventricular depolarization (R waves) were detected through the device software. The time elapsed between R waves (RR intervals) was then computed. We visually identified and manually tagged premature and lost beats in the original file of RR intervals. These abnormal beats were replaced by RR intervals resulting from linear interpolation<sup>5</sup>. Only those segments with >85% qualified beats were included in the analyses<sup>19, 20</sup>.

*Time domain HRV analysis:* Quantitative time series analysis was performed on heart rate by evaluating measures of variation over time. Among these, RRm (mean duration of RR intervals in ms) quantifies the mean heart rate, SDNN (standard deviation of RR intervals in ms) represents a coarse quantification of overall variability, and RMSSD (square root of the mean squared differences of successive normal RR)

measures short-term heart rate variations <sup>5,6</sup>.

*Spectral HRV analysis:* These measurements provide an evaluation of the power (amplitude) of the contributing frequencies underlying HRV <sup>6</sup>. For the analysis of cyclic HRV components the wavelet transform was chosen rather than the traditional Fast Fourier Transform (FFT) because it allowed a better fit of a signal to nonlinear fluctuations. The power of the signal along time was determined at previously defined frequency bands <sup>21</sup>: total area (TA, 0 - 1.2 Hz), very low frequency (VLF, 0 - 0.0375 Hz), low frequency (LF, 0.0375 - 0.15 Hz) and high frequency (HF, 0.15 - 1.2 Hz). Reported values are expressed as the natural logarithm of TA, HF, LF and VLF; normalized for the total power; and as the ratio between LF and HF. Details of this procedure can be found elsewhere <sup>21, 22</sup>.

*Non - linear HRV analysis:* The short term fractal correlation exponent " $\alpha$ s" <sup>8</sup> and the "Sample Entropy" index (SampEn) <sup>23</sup> were used as nonlinear HRV indexes. The scaling exponent  $\alpha$ s, based on the "detrended fluctuation analysis", quantifies the short-term (< 11 beats) fractal correlation properties of the interbeat time data. Values of  $\alpha$ s close to 0.5 are associated with uncorrelated RR intervals, whereas values close to 1.5 are associated with strong correlation between RR intervals. Values near 1 are characteristic of fractal like processes, associated with the dynamic behavior of time series generated by complex systems, such as the autonomic regulation of the sinus rhythm in healthy subjects <sup>8</sup>. SampEn measures the degree of irregularity of the RR interval time series. Regular sequences will result in lower SampEn values whereas random behavior is associated with larger SampEn values. These methods have been previously described <sup>6, 8, 23</sup>.

### *Statistical analysis*

Values are expressed as mean  $\pm$  standard error. Normality of distributions was verified by the Kolmogorov Smirnov test.

Demographic characteristics, time spent in hypoxia, SaO<sub>2</sub> and mean heart rate were compared between included and excluded subjects by means of a Mann Whitney U test.

The HRV indexes obtained during baseline, hypoxia, and recovery periods were compared by means of a repeated measures analysis of variance (ANOVA) followed by a post hoc Bonferroni test to establish intergroup differences.

Additional associations of the time spent in hypoxia with the time spent on 100% O<sub>2</sub> before the test, and with HRV data were analyzed through a Pearson correlation test.

## **Results**

The final sample included 12 subjects, because five individuals had hypoxia periods with less than 100 RR intervals. The demographic characteristics of included and excluded subjects are shown in Table 1.

During the hypoxia period, the included group showed a mean of  $138 \pm 13$  RR intervals (minimum 100, maximum 253), while the excluded group showed a mean of  $70 \pm 3$  RR intervals (minimum 63, maximum 80). The percentage of premature or missed beats was similar in both groups (less than 2% of the data).

The time that the pilots spent with 100% O<sub>2</sub> before the test had no correlation with the time spent in hypoxia. Both included and excluded pilots reported similar hypoxia-related symptoms, including transient visual derangements, flushing in the face or limbs and psychomotor lack of coordination. Table 2 depicts time, SaO<sub>2</sub> and mean heart rate for each stage in included and excluded subjects. When comparing with the included group, the excluded subjects showed a reduced time of hypoxia exposure with similar SaO<sub>2</sub> values and a lower mean heart rate. This justifies that this group has had less than the minimum 100 beats required for HRV analyses.

HRV analysis is shown in Table 3. Linear analysis revealed a marked increase of heart rate related to hypoxia associated to a global decrease of HRV at all frequency ranges. Once oxygen was restored, mean heart rate and global HRV tended to return to basal levels. This trend was not observed in the power spectral indexes of HRV, where the hypoxia period was different from the basal stage but similar to the recovery stage. No significant differences were observed in normalized units of HRV. Nonlinear analysis revealed a marginally significant effect of hypoxia in decreasing  $\alpha_s$  and a significant effect of hypoxia in increasing SampEn. Once oxygen was restored, SampEn value remained significantly different to the basal stage. HRV measurements at basal, hypoxia and recovery stages had no correlation with the time spent in hypoxia.

Figure 1 shows a typical HRV recording along the three epochs considered for analysis (basal, hypoxia and recovery) with the transitions periods among them. Note the increase of heart rate (lower RR interval duration) and the decrease in the amplitude of heart rate oscillations in the hypoxia period (RRm = 594, SDNN = 34 ms), in comparison to basal (RRm = 734 ms, SDNN = 107 ms) and recovery (RRm = 893 ms, SDNN = 55 ms) periods.

To account for differences in the mean and amplitude of heart rate fluctuations, Figure 2 shows the recordings corresponding to each epoch with RR intervals rescaled as standard deviation units centered at a mean of zero. When compared with basal period (SampEn = 0.89), a higher irregularity of the oscillations during hypoxia period (SampEn = 1.61) and afterwards (SampEn = 1.58) could be noted.

## Discussion

The main finding of the present study is that acute severe hypoxia is associated with greater nonlinear HRV, as evidenced by the observed higher values of SampEn, a



measurement of the irregularity of the RR interval time series. This change was accompanied by a decrease of linear HRV of similar proportions in all frequency levels, as previously described by others<sup>2-4, 24</sup>.

The marked fall in oxygen saturation and the appearance of subjective symptoms indicate that the pilots were exposed to considerable hypoxic stress. The present study differs from others in the sudden desaturation and the severity of the hypobaric hypoxia condition<sup>12, 13</sup>. In addition, comparison of hypoxia effects in each of the subjects and at the same simulated altitude reinforces the evidence that the observed changes were caused by hypoxemia.

Changes observed in the linear components of HRV could be related to the increase of heart rate due to the hypoxia effect, as the amplitude of the oscillations of different frequencies as well as the length of the RR interval diminished<sup>25</sup>. However, changes observed in nonlinear components of HRV may not be directly related to the changes in heart rate, especially when heart rate is high<sup>25</sup>. In fact, physical exercise has been associated to either decreased<sup>7</sup> or increased nonlinear HRV<sup>10</sup>. It was shown that during incremental exercise test  $\alpha$ s increased from rest up to an intensity level of approximately 40% of maximum oxygen consumption, declining thereafter linearly towards values that reflect no correlation between RR intervals<sup>10, 26</sup>. This pattern can be explained by the varying intensity of sympathetic input to the sinus node during different intensity levels of exercise<sup>26</sup>. Reduced values of  $\alpha$ s are usually associated with increased values of SampEn<sup>25</sup>, as seen in the present study. The extreme activation of the sympathetic nervous system underlying severe hypoxia and vigorous exercise may lead to an increase in propensity to ventricular fibrillation<sup>14, 27, 28</sup>. Thus, the common HRV pattern of severe hypoxia described herein and the one of vigorous exercise described by others<sup>10, 26</sup> might reflect this increased risk.

An alternative explanation for the observed changes of nonlinear HRV dynamics after hypoxia is that they are related to intrinsic cardiac effects resulting from compensatory physiologic mechanisms induced by hypoxia. It is known that pathological disruption of specific components of the intrinsic cardiac plexus can compromise the autonomic control of the heart, thus causing arrhythmogenesis<sup>29</sup>. It has been proposed that this system would act as a "low pass filter", to smooth the effect of spurious extracardiac stimuli and to maintain a balance between the electrical and mechanical functions of the heart by means of local feedback circuits<sup>30</sup>. A functional alteration of this "filter" in normal subjects under hypoxia could produce the observed pattern of greater nonlinear irregularity which remained elevated even after oxygen was restored. In any case, an alteration of the complexity of dynamics of heart rate fluctuations as described in the present work can render the heart less adaptable for the changing environmental requirements or may start transient electric alterations favoring the appearance of arrhythmias<sup>29, 30</sup>. In this respect, it is important to note that our results are similar to the nonlinear HRV pattern described in ischemic heart disease patients with high risk for fatal arrhythmias<sup>11</sup>.

Some limitations of the present study must be taken into account. The hypoxia period is a non-spontaneous and non-resting state. A maximal sympathetic activation and vagal withdrawal allowed the cardiovascular system to achieve a maximal constant cardiac output during a defined period of time, after which all variables rapidly decline

towards pre hypoxic levels. Thus, even if the hypoxia state is unstable, we can assume that the data were stationary in the temporal window considered<sup>31</sup>. In this regard, transition periods between states were not considered for analysis and data were detrended to avoid possible trends. A further limitation is related to the application of nonlinear HRV indexes to short sets of HRV data. However, it was reported that the application of nonlinear indexes such as SampEn or ApEn to 100 data points series, yield statistically reliable and reproducible results<sup>6, 23</sup>. Regarding the use of the short term scaling exponent  $\alpha_s$ , it was found that, from a collective point of view, there is statistical agreement in real data between the means of the exponents derived from long and short segments involving the use of 100 data points<sup>18</sup>.

In sum, this study shows that severe acute hypoxia is associated with a pattern of diminished linear HRV and increased nonlinear HRV, different from what has been seen in previous studies at lower altitudes. While this pattern is similar to that observed during vigorous exercise or in patients with ischemic heart disease in risk for fatal arrhythmias, future studies are needed to assess whether it is related with an increased risk of sudden death in healthy people.

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## TABLES

**Table 1.** Subjects characteristics

	Included subjects (n = 12)	Excluded subjects (n = 5)	p
Age (years)	28 ± 1	27 ± 3	0.915
Weight (kg)	78 ± 4	76 ± 3	0.874
Height (m)	1.76 ± 0.02	1.75 ± 0.02	0.596
BMI (kg/m <sup>2</sup> )	25.2 ± 1.2	24.8 ± 0.7	0.752

Values are presented as mean ± standard error. Intergroup differences were assessed by means of Mann - Whitney U test. BMI: body mass index.

**Table 2.** Stage characteristics in included and excluded subjects

Stage	Included subjects (n = 12)			Excluded subjects (n = 5)		
	Time (s)	SaO2 (%)	Heart rate (bpm)	Time (s)	SaO2 (%)	Heart rate (bpm)
Basal	89 ± 9 <sup>a</sup>	98.3 ± 0.3	94 ± 4	51 ± 2 <sup>a</sup>	98.2 ± 0.6	84 ± 3
Transition	43 ± 4	-	-	57 ± 12	-	-
Hypoxia	70 ± 6 <sup>b</sup>	I: 91.7 ± 1.3 F: 64.8 ± 2.7	120 ± 5 <sup>d</sup>	43 ± 1 <sup>b</sup>	I: 90.8 ± 1.9 F: 65.8 ± 2.7	95 ± 6 <sup>d</sup>
Transition	37 ± 2	-	-	47 ± 6	-	-
Recovery	100 ± 12 <sup>c</sup>	97.9 ± 0.5	86 ± 4 <sup>e</sup>	65 ± 3 <sup>c</sup>	97.2 ± 0.9	65 ± 2 <sup>e</sup>

Values are presented as mean ± standard error. Differences between included and excluded subjects were assessed by means of a Mann - Whitney U test. Significant differences were found for the time covered by each stage (a: p = 0.004; b: p = 0.003; c: p = 0.008) and for the mean heart rate in the hypoxia and recovery stages (d: p = 0.027; e: p = 0.004). I: initial and F: final SaO2 of the hypoxia stage. See the text for details.

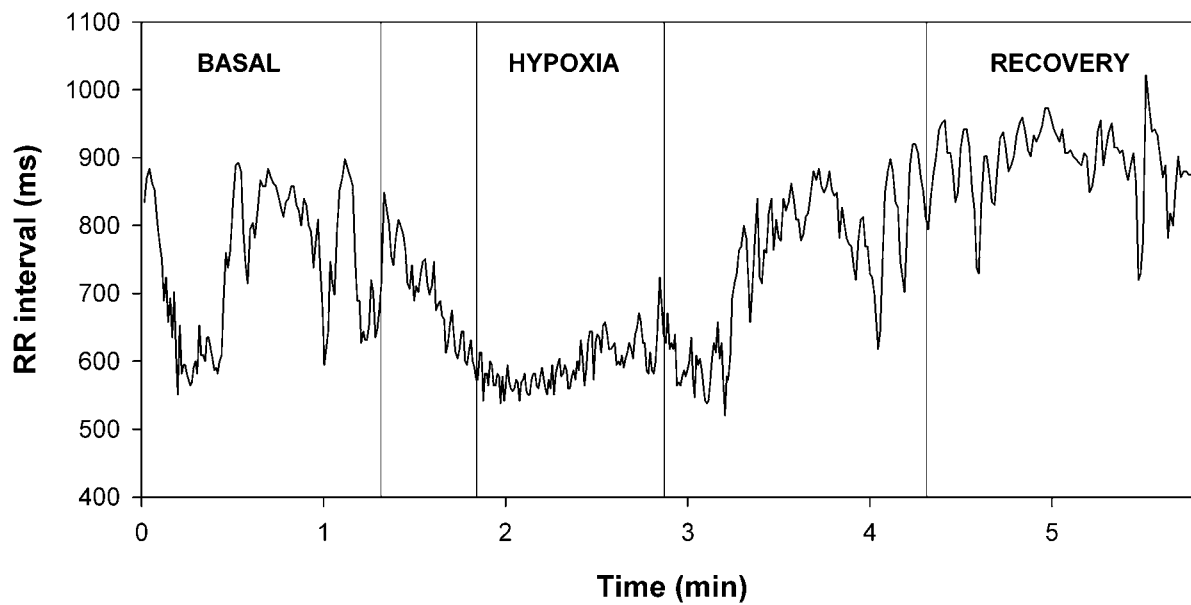
**Table 3.** Heart rate variability analysis.

	basal	hypoxia	recovery	p
Heart rate (bpm)	94 ± 4	120 ± 5 <sup>d</sup>	86 ± 4 <sup>e</sup>	< 0.001
RRm (ms)	638 ± 30	509 ± 21 <sup>a</sup>	716 ± 32 <sup>a</sup>	< 0.001
SDNN (ms)	71 ± 11	25 ± 6 <sup>a</sup>	44 ± 5 <sup>a</sup>	0.002
RMSSD (ms)	30 ± 4	21 ± 5	27 ± 4	0.140
ln TA (ms <sup>2</sup> )	11.9 ± 0.3	11.0 ± 0.3 <sup>b</sup>	11.2 ± 0.2 <sup>b</sup>	< 0.001
ln VLF (ms <sup>2</sup> )	11.6 ± 0.3	10.6 ± 0.4 <sup>b</sup>	10.8 ± 0.2 <sup>b</sup>	0.001
ln LF (ms <sup>2</sup> )	10.5 ± 0.3	9.6 ± 0.4 <sup>b</sup>	9.9 ± 0.3 <sup>b</sup>	0.009
ln HF (ms <sup>2</sup> )	8.3 ± 0.2	7.7 ± 0.4 <sup>b</sup>	8.0 ± 0.3	0.046
VLF (%)	72.8 ± 2.7	66.7 ± 4.1	66.4 ± 2.7	0.160
LF (%)	24.1 ± 2.3	28.6 ± 3.7	29.1 ± 2.3	0.234
HF (%)	3.1 ± 0.5	4.6 ± 0.7	4.5 ± 0.5	0.068
LF/HF	9.6 ± 1.4	6.6 ± 0.6	7.6 ± 1.0	0.095
αs	1.39 ± 0.07	1.11 ± 0.13	1.41 ± 0.05	0.054
SampEn	1.07 ± 0.11	1.45 ± 0.12 <sup>b</sup>	1.43 ± 0.09 <sup>b</sup>	0.018

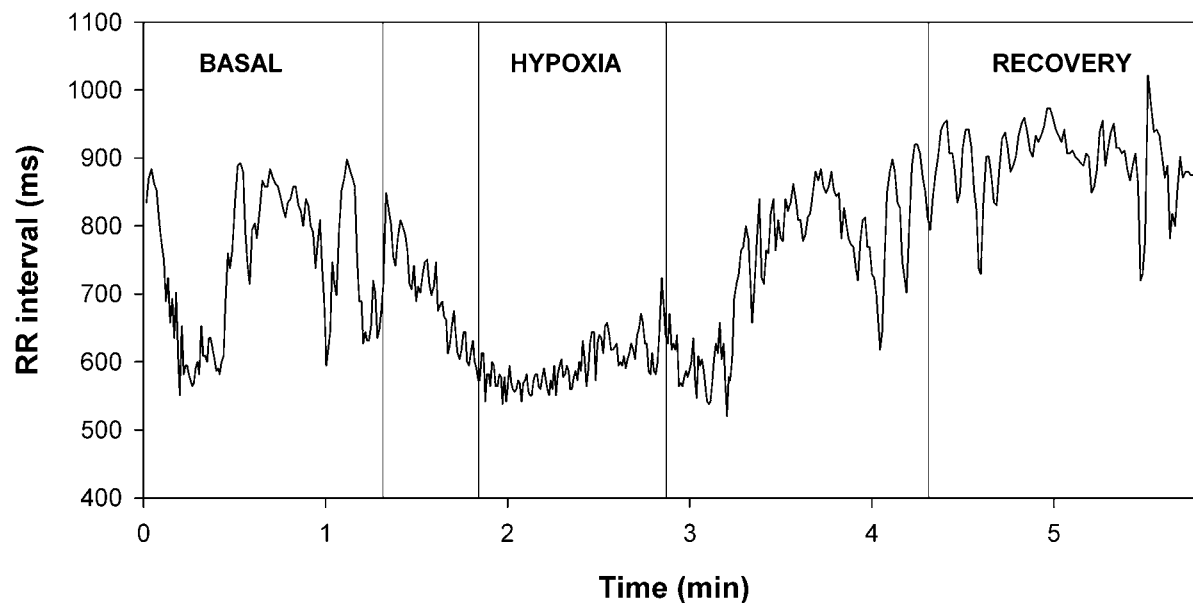
Values are expressed as mean ± standard error. <sup>a</sup> Different from each other, <sup>b</sup> different from basal. ANOVA repeated measures followed by Bonferroni test. RRm, mean of RR interval duration; SDNN, standard deviation of RR intervals; RMSSD, square root of the mean squared differences of successive normal RR; TA, total area power; VLF, very low frequency power; LF, low frequency power; HF, high frequency power; αs, short term fractal correlation exponent; SampEn, sample entropy. See the text for details.



## Figures legends



**Figure 1:** RR interval recording of one subject along the three epochs considered for analysis, with the transitions periods between them.



**Figure 2:** RR interval recording corresponding to basal, hypoxia and recovery periods of the same subject of figure 1. To account for differences in the mean and amplitude of heart rate fluctuations, RR intervals were rescaled as standard deviation units centered around a mean of zero.