Study of heart rate variability following exposure to normobaric hypoxia

Inés Albertus Cámara, María José Paredes Ruiz, María Jódar Reverte, Vicente Ferrer López, Ignacio Martínez González-Moro

Grupo de Investigación Ejercicio Físico y Rendimiento Humano. Campus Mare Nostrum. Universidad de Murcia.

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Summary

Heart rate variability (HRV) is a tool capable of analysing and assessing the vegetative activity of the heart in various activities and situations. It consists of measuring the time that elapses between every two heartbeats over a period of time and expressing it in terms of mathematical and statistical equations. Other authors have analysed the influence of different stressors on HRV. In this work we are looking for the action of normobaric hypoxia (NH) on HRV. NH consists of breathing oxygen-depleted air simulating altitude training. The aim of the study is to determine the influence of HN on the time and frequency domains of HRV. We subjected 13 healthy subjects (recreational athletes) to two HN sessions. We used the iAltitude Trainer v2.7® simulator. The first was a hypoxia tolerance test (HTT) (10 minutes, 11% O_2, equivalent to 5050m) and the second was an intermittent exposure (HNI) (14% O_2, 3250m) in which periods of 4 minutes of hypoxia alternated with 4 minutes of normoxia for 64 minutes. For HRV analysis, a Polar H10® heart rate monitor, the HRV-elite® application and the Kubios-Standard® software were used. Data were taken 5 minutes before and after each session, and these values were compared using the Student´s t- test for paired data. None of the variables in the time (RRmean, SDNN, rMSSD, pNN50) or frequency (VLF, LF, HF, LF/HF) domains of HRV showed significant changes in either situation. HN did not cause changes in the stress levels of these subjects and was well tolerated, clinically and electrocardiographically. A tolerance test and a session of exposure to intermittent normobaric hypoxia are not sufficient stimuli to cause acute changes in HRV.

Key words:
Heart rate variability. Intermittent normobaric hypoxia. Hypoxia tolerance.

Estudio de la variabilidad de la frecuencia cardiaca tras la exposición a la hipoxia normobárica

Resumen

La variabilidad de la frecuencia cardiaca (VFC) es una herramienta capaz de analizar y valorar la actividad vegetativa sobre el corazón ante diversas actividades y situaciones. Consiste en medir el tiempo que trascurre entre cada dos latidos cardiacos durante un periodo de tiempo y expresarlo en función de ecuaciones matemáticas y estadísticas. Otros autores han analizado la influencia de diferentes estresores sobre la VFC. En este trabajo buscamos la acción de la hipoxia normobárica (HN) sobre la misma. La HN consiste en respirar aire empobrecido de oxígeno simulando el entrenamiento en altitud. El objetivo del estudio es determinar la influencia de la HN sobre los dominios de tiempo y frecuencia de la VFC. Sometimos a 13 sujetos sanos (deportistas recreacionales) a dos sesiones de HN. Usamos el simulador iAltitude Trainer v2.7®. La primera mediante un test de tolerancia a la hipoxia (TTH) (10 minutos, 11% O_2, equivalente a 5.050 m) y, la segunda, con una exposición intermitente (HNI) (14% O_2, 3.250 m) en la que se alternaron periodos de 4 minutos de hipoxia con 4 de normoxia durante 64 minutos. Para el análisis de VFC se utilizó un pulsómetro Polar H10®, la aplicación HRV-elite® y el software Kubios-Standard®. Se tomaron los datos de los 5 minutos previos y posteriores a cada sesión, comparándose estos valores mediante el test de T-student para datos pareados. Ninguna de las variables de los dominios de tiempo (RRmedio, SDNN, rMSSD, pNN50) ni de frecuencia (VLF, LF, HF, LF/HF) de la VFC mostró cambios significativos ante ninguna de las dos situaciones. La HN no provocó modificaciones en los niveles de estrés de estos sujetos, siendo bien tolerada, clínica y electrocardiográficamente. Un test de tolerancia y una sesión de exposición a hipoxia normobárica intermitente no son estímulos suficientes para provocar cambios agudos en la VFC.

Palabras clave:
Variabilidad de la frecuencia cardiaca. Hipoxia normobárica intermitente. Tolerancia a la hipoxia.

Correspondence: Inés Albertus Cámara
E-mail: ines.albertusc@gmail.com

Introduction

Heart rate variability (HRV) has been defined as the variation in the time interval between consecutive heartbeats, termed R-R intervals[1]. HRV can be assessed through linear methods, analysed through time and frequency domains, and non-linear methods[2,3]. In the time domain, the following statistical indices are highlighted: Mean R-R (mean of the R-R intervals); SDNN (standard deviation of the normal-to-normal intervals; RMSSD (root mean square of successive RR interval differences); pNN50 (total percentage of successive RR intervals that differ by more than 50 ms); and, in the frequency domain: VLF (very low frequency); HF (high frequency); LF (low frequency); LF/HF (ratio of low frequency to high frequency)[4-6].

Traditionally, HRV has been used in the area of sports for the purpose, among others, of improving the adaptation or performance of athletes[7]. Apart from sport, it has other uses in the field of medicine, given that it has been used in different pathologies such as cardiac[8], anxious depression[9] and currently in the COVID-19 disease as a potential acute inflammation marker [10] or as a method to detect the effects triggered by lockdown on this variable[11].

Many and varied factors can influence heart rate and, therefore, they can also have an influence on the analysis of heart rate variability. These factors can be divided into intrinsic or extrinsic to the body. With regard to the former, the following are established: age (HR decreases with age)[12], sex (in general, HR is greater in women)[13], body position (HR is lower in the supine position)[14] and work-related stress[15]. With regard to extrinsic factors, of particular note are: humidity[16], the time of day at which the measurement is taken (HR is higher in the early morning)[14], ambient temperature[17] or the intake of substances such as caffeine[18,19]. One stimulus that has been little investigated is the influence of normobaric hypoxia (breathing air with a reduced oxygen fraction at constant atmospheric pressure) on HRV.

With increased altitude, the barometric pressure drops exponentially, giving rise to a progressive reduction in the partial pressure of oxygen (pO_2) in the atmosphere, this type is referred to as hypobaric hypoxia[20]. However, with technological progress, altitude simulators have been developed that make it possible to reduce the amount of oxygen in the air without altering the atmospheric pressure, giving rise to what is known as normobaric hypoxia (HN)[21].

Training in places with a reduced oxygen fraction has been a particularly important resource in the area of sport. It can represent a stimulus for the body, improving the hypoxia adaptation systems and, consequently, acting as a supercompensation mechanism[22].

Depending on the Hypoxia exposure time, it is possible to differentiate between chronic exposure, made over long periods of time, and acute exposure and, within the latter, intermittent normobaric hypoxia (INH) which consists in applying periods of hypoxia followed by periods of normoxia (normal oxygen fraction).

The aim of this study is to determine the variations arising in the domains of heart rate variability following exposure to normobaric hypoxia.

Material and method

This investigation is a prospective intervention study. A non-probability convenience selection method was used to select the participants. Each participant signed an informed consent prior to data collection. This consent stated the study objectives, the conditions in which the measurements would be taken, the confidentiality and security of the information obtained. Prior to the investigation, approval was obtained from the Ethics Committee of Investigation of the University of Murcia. In all cases, the study met the requirements of the Code of Ethics of the World Medical Association (Declaration of Helsinki) for trials with human subjects.

Participants

Our study comprised thirteen subjects (53.3% women). The age of the participants ranged between 20 and 29 years. They did not suffer from any prior cardiac or respiratory disease.

The inclusion criteria were as follows: 1) Express a willingness to participate in the study, having understood the scope of the same, the risk and benefits of the intervention, confirming this willingness to participate by signing the informed consent. 2) Be aged between 20 to 30 years. Excluded from the study were those with pathologies that contraindicated the testing; the presence of a body temperature of more than 37ºC and/or a positive antibody test. 3) Have had the Covid-19 disease and, at the time of the study, have no negative PCR tests or not completing either of the two hypoxia tests for reasons not related to the tests.

Instruments

For the anthropometric study, the height (SECA 213®) and weight (In Body 120®) were recorded. The waist and thigh circumferences were obtained with a Holtain® flexible metal tape measure and a Lux® device was used to measure the blood cell count. The blood pressure and auscultation were taken with a conventional stethoscope and sphygmomanometer (Littmann Clasic®).

The tolerance test and the training session were conducted with a Hypoxia simulator, namely the iAltitude Trainer v2.7® connected to a specific mask. During both tests, all participants were monitored for muscle oxygenation through the Humon Hex® device in the anterior rectus muscle of the right thigh, for oxygen saturation with a pulse oximeter (Nonin®) in the left ear lobe, and heart rate variability with a Polar H10® heart rate monitor.

Furthermore, due to the special circumstances resulting from COVID-19, temperature was taken using the Yuwell® digital thermometer and the 2019-nCoV IgG/IgM®" antibody test was used to rule out the presence of the SARS-CoV-2 virus.

Prior procedure

After voluntarily signing the informed consent, each participant underwent the measurement of the different anthropometric parameters followed by a medical examination to rule out any alteration that could contraindicate the performance of the tests. The prior procedures and both hypoxia tests were conducted at the Biomedical Research Laboratory (LAIB) of the University of Murcia. The room temperature was always constant at 25 ºC.

The body temperature was taken first, this was followed by the antibody test, the determination of the red blood cell count and the anthropometric study. The participant then completed a questionnaire on diseases and/or family history. This was checked by a physician through a direct interview.

Secondly, the blood pressure was taken in a supine position, the participant was auscultated and an electrocardiogram at rest was performed (Figure 1).

Performance of the hypoxia tests

Having checked for the absence of pathologies that could contraindicate the test, the participant was then subjected to breathing in thin air while continuously monitoring heart rate variability. A tolerance test was first performed to check the adaptation of the individual to hypoxia before conducting the hypoxia exposure session. In both tests, if the hypoxia simulator detected arterial oxygen saturation values of less than 83%, then it indicated the withdrawal of the mask through visual and audio signals, thereby breathing in normoxic conditions and recovering the normal concentration of oxygen.

For both tests, the participants were in a seated position in an armchair, allowing them to rest their heads and flex their hips and knees 90º. A foam rubber wedge was positioned to give correct back support, giving a comfortable and relaxed position (Figure 2). Participants wore a Polar H10® chest strap linked to a tablet by Bluetooth connection and data were recorded through the HRV elite® application.

Figure 1. Prior examination of a participant.

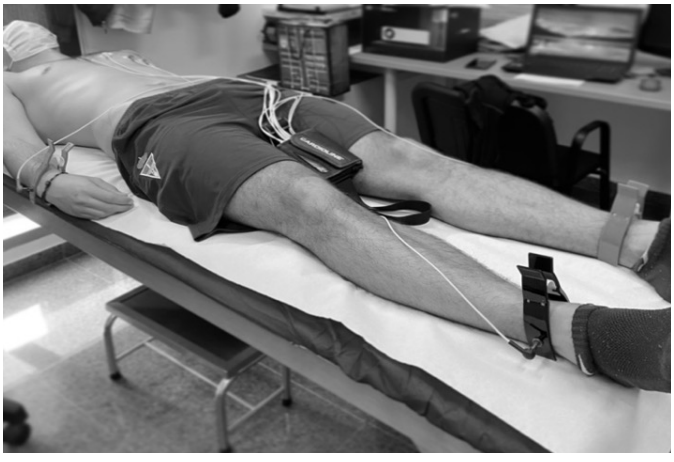

Figure 2. A participant performing a hypoxia test.

Hypoxia tolerance test

In the position described above, the subject remained seated and relaxed for 5 minutes before the start of the tolerance test to record their HRV at rest and in normoxia.

The tolerance test was then conducted. It consisted in continuously breathing in under hypoxic conditions (11% oxygen, equivalent to an altitude of 5,050 m) for a maximum time of 10 minutes. Once the test had ended, the subject remained seated in order to record the 5 minutes subsequent to the completion of the tolerance test.

Therefore, two measurements of the HRV were taken, both under normoxic conditions The first reading shows the five minutes prior to subjecting the subject to the hypoxia tolerance test while the second one records the five minutes subsequent to the completion of this test.

Exposure session

Once the tolerance test had been completed, the volunteer remained seated in the position described above in order to start the second hypoxia test. The heart rate variability was recorded during the 5 minutes prior to the test. When this time was up, the intermittent hypoxia exposure session commenced. The duration of the session was 64 minutes, with 14% oxygen (equivalent to an altitude of 3,250 m). The intermittent mode was applied. In other words, a period of hypoxia (4 minutes) was followed by a normoxia period (4 minutes) alternating until the completion of the 64-minute session. At the end of the session, the sunbsequent 5 minutes were recorded in order to analyse the HRV.

Therefore, two measurements of the HRV were taken, both under normoxic conditions The first record corresponds to the 5 minutes prior to the start of the hypoxia exposure session and the second record to the period immediately after completion of the session. Both records had a 5-minute duration.

Statistical analysis

Based on the data from the different computer applications and software, a spreadsheet was prepared using the Excel® program and, in turn, the data were analysed with the SPSS 24.0® statistical software.

The quantitative variables were described through the mean and standard deviation. The Coefficient of Variation was used to check the data dispersion. The homogeneity of variance was determined with the Levene test. Intra-individual comparisons were found through the paired T-test and the inter-group comparisons with the T-student test. Significant differences or relationships were considered to occur when $p<0.05$.

Results

The study population comprised 6 males and 7 females. Table 1 describes the characteristics of the population in general and separated by sex. Significant differences were observed ($p<0.05$) between sexes in the variables for height, weight and waist circumference, obtaining higher values in the male study population.

When comparing the HRV values prior and subsequent to the performance of the tolerance test, no significant differences were found in any of the domains: time (Table 2) and frequency (Table 3).

Table 1. Characteristics of the overall population and separated by sex.

Variable	Population	Mean	SD	CV (%)	t	Sig. (bilateral)
Age (years)	Overall (n = 13)	23	2.58	11.22		
	Males (n = 6)	22.0	1.26	5.73	-1.41	0.194
	Females (n = 7)	23.86	3.19	13.37		
Height (cm)	Overall (n = 13)	173.75	9.24	5.32		
	Males (n = 6)	182.18	4.97	2.72	6.129	0.000
	Females (n = 7)	166.51	4.25	2.55		
Weight (kg)	Overall (n = 13)	72.72	12.80	17.60		
	Males (n = 6)	83.21	5.27	6.33	4.270	0.001
	Females (n = 7)	63.72	10.01	15.71		
Waist C.(cm)	Overall (n = 13)	77.02	8.33	10.82		
	Males (n = 6)	83.21	4.99	6.00	3.411	0.006
	Females (n = 7)	71.71	6.82	9.51		
Hip C. (cm)	Overall (n = 13)	98.13	6.40	6.52		
	Males (n = 6)	101.11	4.69	4.63	1.667	0.124
	Females (n = 7)	95.57	6.87	7.19		
BMI (kg/m^2)	Overall (n = 13)	23.94	2.74	11.45		
	Males (n = 6)	25.08	1.44	5.74	1.45	0.176
	Females (n = 7)	22.97	3.30	14.37		

Waist C.: waist circumference; Hip C.: hip circumference; CV: coefficient of variation; SD: standard deviation; Sig. (bilateral): bilateral signification.

Table 2. Differences in the time domain component of heart rate variability before and after the tolerance test (n=13).

Time domain variables	Mean	SD	Mean of the differences	SD of the mean	t	Sig. (bilateral)
Start RRmean	869.35	143.16	4.20692	60.03661	0.253	0.805
End RRmean	865.14	109.06				
Start SDNN	73.21	21.19	-6.59908	24.80853	-0.959	0.356
End SDNN	79.81	35.09				
Start RMSSD	50.40	23.73	1.11462	11.52709	0.349	0.733
End RMSSD	49.29	24.03				
Start pNN50	26.09	19.76	1.56176	9.44434	0.596	0.562
End pNN50	24.53	19.70				

RRmean: mean of RR intervals; SDNN: Standard deviation of normal-to-normal intervals; RMSSD: root mean square of successive RR interval differences; pNN50: percentage of successive RR > 50 ms; ST: standard deviation.

Table 3. Differences in the frequency domain component of heart rate variability before and after the tolerance test (n=13).

Frequency domain variables	Mean	SD	Mean of the differences	SD of the mean of the differences	t	Sig. (bilateral)
Start VLF	2299.84	1664.02	-1866.11	5979.313	-1.125	0.282
End VLF	4165.95	5925.52				
Start LF	2341.87	1972.07	-25.82077	1580.123	-0.059	0.954
End LF	2367.69	2039.74				
Start HF	980.11	668.23	29.19838	249.249	0.422	0.68
End LF	950.91	716.12				
Start LF/HF	3.05	2.45	-0.3728	1.487	-0.904	0.384
End LF/HF	3.42	2.39				

VLF: very high frequency; HF: high frequency; LF/HF: low frequency to high frequency ratio; LF: low frequency; SD: standard deviation

During the intermittent hypoxia session, no significant differences were obtained when comparing the values of the time domain

prior to the INH session with those obtained at the end of the same (Table 4). Neither were significant differences (p<0.05) found in the frequency domain (Table 5).

Table 4 Differences in the time domain component of heart rate variability before and after the Hypoxia session (n=13).

Variables	Mean	SD	Mean of the differences	SD of the mean of the differences	t	Sig. (bilateral)
Start RRmean	854.55	106.70	36.47	58.58	2.157	0.054
End RRmean	891.02	120.05				
Start SDNN	81.74	35.92	-1.73	18.81	-0.319	0.756
End SDNN	80.01	24.45				
Start RMSSD	47.09	23.70	0.134	8.83	0.055	0.957
End RMSSD	47.23	17.47				
Start pNN50	21.48	17.06	2.25	5.44	1.436	0.179
End pNN50	23.73	15.,72				

RRmean; mean of the RR intervals; SDNN: Standard deviation of normal-to-normal intervals; RMSSD: root mean square of successive RR interval differences; pNN50: percentage of successive RR > 50 ms; ST: standard deviation.

Table 5 Differences in the frequency domain of heart rate variability before and after the INH exposure session (n=13).

Variable	Mean	SD	Mean of the differences	SD of the mean of the differences	t	Sig. (bilateral)
Start VLF	3085.77	2073.66	349.61	3059.15	0.396	0.700
End VLF	2736.16	3362.20				
Start LF	2289.60	1708.52	-213.92	1068.16	-0.694	0.502
End LF	2503.52	2068.12				
Start HF	731.98	461.18	-146.22	482.91	-1.049	0.317
End HF	878.19	696.04				
Start LF/HF	3.43	2.62	-0.24	1.67	-0.505	0.623
End LF/HF	3.67	2.31				

VLF: very high frequency; HF: high frequency; LF/HF: low frequency to high frequency ratio; LF: low frequency; SD: standard deviation.

Discussion

This study analysed the heart rate variability response following a tolerance test and a session with exposure to normobaric hypoxia. It has been demonstrated that hypoxia, at the altitude and temperature described, does not cause significant changes in the HRV domains for time and frequency.

The population of this study comprised 13 subjects, a size that is in concordance with that of other authors[23-25]. The same is true for the mean age of the subjects (23±2.58 years) being very similar to that described in other studies[24,26]. This could be due to the fact that, at present, there are few studies that analyse this phenomenon and, as a prior step, it was conducted on young, healthy individuals so that it will be possible to perform it in the future on other populations subject to greater risks, thereby ensuring that studies are conducted safely.

The altitude simulated and the exposure time used were different depending on whether a tolerance test was being conducted or a session with exposure to hypoxia. In our case, during the tolerance test, an altitude of 5,050 metres (11% O_2) was simulated for a maximum period of 10 minutes, while Buchheit *et al*[23] subjected their participants to a slightly lower altitude at 4,800 metres (11.5% O_2) during this test. However, the protocol used by these authors differs from ours, given that they alternated periods of hypoxia at rest with hypoxia during physical exercise, making a comparison with our study difficult.

Heart rate variability in the time and frequency domains

We have verified, through other studies, how the HRV response can vary depending on whether it is: a short exposure[23,25-27] or more prolonged exposure to hypoxia[28,29]; the type of hypoxic administration, in other words, gradually[29-31] or suddenly[23,25]; the altitude intensity: high[23,29,31], moderate[24] or low[30]; as well as the different types of hypoxia used: normobaric[23,26] or hypobaric[31,25], given that the body's response to each one is different and makes comparison difficult. For this reason, it is important to determine which duration, intensity and type of hypoxia favourably influence HRV

Some authors agree that exposure to normobaric hypoxia induces a decrease of the RMSSD and an increase in the LF/HF ratio, in other words, a greater activation of the sympathetic nervous system[25,32] while other authors uphold an increase in the parasympathetic nervous system[24,31]. However, the tolerance test conducted in our study showed no significant differences in the HRV time and frequency domains. This finding could corroborate the point that other authors are suggesting, in that, in order to induce significant changes in some domains, such as the LF/HF ratio, a prolonged duration of at least 30 minutes is necessary[33]. However, Botek *et al*[26] specified a 10-minute exposure, but with the difference that their participants were subjected to an altitude of 6,200 m (9.6% O), which was higher than the one described in the literature. These authors[26] obtained a decrease in LF and an increase in HF.

Therefore, it appears that, while in 2001 Bernardi *et al*[33] indicated that a 30-minute exposure to hypoxia was necessary to generate changes in the HRV domains, years later Botek *et al*[26] managed to influence the HRV domains by maintaining acute exposures (10 minutes) while increasing the altitude stimulus. For this reason, our study demonstrates that an altitude of 5,050 metres for 10 minutes is not sufficient to affect the HRV, meaning that it would be necessary to further reduce the amount of oxygen inspired.

During the training session, the exposure time was more prolonged (64 minutes) than for the tolerance test, yet reducing the altitude and applied intermittently (intermittent normobaric hypoxia). The 3,250 metres at which the second hypoxic test was conducted in this study may be insufficient to generate changes in heart rate variability despite the fact that exposure duration was longer. In this way, these findings are in line with Yamatho *et al*[34], who also found no significant changes in their study. These authors[34] predicted that an altitude of less than 3,500 metres was not sufficient, a hypothesis which supports the findings of our present study.

Some studies used exposure to hypoxia simultaneously with the practice of physical exercise[34,35]. In this case, there does appear to be more consensus with regard to a greater variation in the absolute time and frequency domains than if hypoxia is administered at rest. However, this may be due to the actual stimulus of physical exercise and not to hypoxia. For this reason, we consider that there is a need to clarify what times and altitudes are necessary in order to generate a beneficial effect on heart rate variability before synchronously including a further stimulus such as physical exercise.

Conclusion

Heart rate variability shows no significant changes neither in the time domain nor in the frequency domain following exposure to normobaric hypoxia. We therefore consider that these stimuli were not sufficiently stressful to cause acute changes.

Conflict of interest

The authors have no conflict of interest at all.

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