Apunts Med Esport. 2016;**51(190)**:40-47

apunts
MEDICINA DE L'ESPORT
www.apunts.org

ORIGINAL ARTICLE

Effect of rarefied air in a Mediterranean cave on human cardiovascular function

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Received 2 November 2015; accepted 18 January 2016

KEYWORDS
Rarefied air;
Hypoxia;
CO_2;
Parasympathetic;
Heart rate variability;
Arrhythmia

Abstract

Introduction: Study of physiological adaptation in people breathing rarefied air in a cave.
Objective: To investigate the arrhythmogenic capacity of rarefied air and changes the autonomic nervous system (sympathetic and parasympathetic). To establish cutoff levels beyond which preventive measures must be taken.
Method: The study included 25 cavers, monitored by ECG Holter and blood pressure measurements in 2 situations at rest, one outside the cave breathing normal air composition (NA), and the other underground, breathing rarefied air of natural origin (RA) in a confined space (O_2: 13.38 ± 1.5% and CO_2: 2.23 ± 0.31%).
Results: Resting heart rate (NA: 81.9 ± 15.1 beats per minute [bpm] vs. RA: 83.8 ± 17.3 bpm; $P \leq .58$). Systolic blood pressure (NA: 130.3 ± 17.2 mmHg vs. RA: 140.2 ± 21.3 mmHg; $P \leq .0003$).Diastolic blood pressure (NA: 78.2 ± 11.0 mmHg vs. RA: 85.5 ± 11.2 mmHg; $P \leq .0002$). Heart rate variability: RMSSD (NA: 25.9 ± 13.8 ms vs. RA: 36.9 ± 17.8 ms; $P \leq .003$), NN50 (NA:49.0 ± 66.2 bpm vs. RA: 111.7 ± 102.8 bpm; $P \leq .003$); pNN50 (NA: 11.3% ± 7.5 vs. RA: 15.9 ± 15.8%; $P \leq .0013$). Fourier analysis: TP (NA: 1,759.5 ms^2 vs. RA: 2,611.5 ms^2; $P \leq .04$); HF (NA: 301.5 ± 329.4 ms^2 vs. RA: 662.3 ± 762.8 ms^2; $P \leq .02$). An increase in arrhythmic events is detected when comparing the hour that included test 1 (HNA) in normal air with the hour that included test 2 (HRA) with rarefied air. There is a correlation of arrhythmic events in both situations: (ventricular ectopic beats in RA) = 2.9859 × (ventricular ectopic beats NA) + 1.5622; n = 24; r = 0.814; $P < .0001$.
Conclusions: Exposure to RA at rest for 10 minutes causes a pressor response in systolic and diastolic blood pressure compared to normal air (NA). Heart rate variability in a

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standardized situation and rest shows a parasympathetic response, with increased rMSSD and HF parameters when subjects are subjected to an atmosphere of RA. In RA, the subjects had three times more arrhythmic events when compared to NA.

PALABRAS CLAVE
Aire enrarecido;
Hipoxia;
CO_2;
Parasimpático;
Variabilidad cardíaca;
Arritmia

Impacto del aire enrarecido de una cueva mediterránea en humanos, a nivel cardiovascular

Resumen

Introducción: Estudio de la adaptación fisiológica en personas respirando aire enrarecido en una sima.
Objetivo: Investigar la capacidad arritmogénica del aire enrarecido y las alteraciones del sistema nervioso autónomo (simpático y parasimpático). Establecer unos niveles de corte más allá de los cuales hay que tomar medidas preventivas.
Método: Veinticinco espeleólogos, 6 de ellos pertenecientes a cuerpos profesionales de rescate, sometidos a controles en reposo en el exterior, respirando aire de composición normal (NA), y un control subterráneo, también en reposo, respirando aire enrarecido de origen natural (RA) en un espacio confinado (O_2: 13,38 ± 1,5% y CO_2: 2,23 ± 0,31%). Monitorizados mediante control Holter cardíaco y presión arterial.
Resultados: Pulso cardíaco de reposo (NA: 81,9 ± 15,1 latidos vs. RA: 83,8 ± 17,3 latidos en RA; $p \leq 0,58$). Presión arterial sistólica (NA: 130,3 ± 17,2 mmHg vs. RA: 140,2 ± 21,3 mmHg; $p \leq 0,0003$). Presión arterial diastólica (NA: 78,2 ± 11,0 mmHg vs. RA: 85,5 ± 11,2 mmHg; $p \leq 0,0002$). Variabilidad cardíaca: RMSSD (NA: 25,9 ± 13,8 ms vs. RA: 36,9 ± 17,8 ms; $p \leq 0,003$); NN50 (NA: 49,0 ± 66,2 latidos vs. RA: 111,7 ± 102,8 latidos; $p \leq 0,003$); pNN50 (7,5 ± 11,3% en NA vs. 15,9 ± 15,8% en RA; $p \leq 0,0013$). Análisis de Fourier: TP (NA: 1.759,5 ms^2 vs. RA: 2.611,5 ms^2; $p < 0,04$); HF (NA: 301,5 ± 329,4 ms^2 vs. RA: 662,3 ± 762,8 ms^2; $p \leq 0,02$). Se detecta un incremento de los acontecimiento arrítmicos cuando comparamos la hora que incluye la prueba 1 (HNA) con aire de proporciones normales versus la hora que incluye la prueba 2 (HRA) con aire enrarecido. Hay una clara correlación estadística de eventos arrítmicos en ambas situaciones: (latidos ectópicos en RA) = 2,9859 × (latidos ectópicos en NA) + 1,5622; n = 24; r = 0,814; $p < 0,0001$.
Conclusiones: La exposición al aire enrarecido en reposo de tan solo 10 min provoca una respuesta presora de la presión arterial sistólica y diastólica, comparada con aire normal. La variabilidad cardíaca, en situación de reposo estandarizada, muestra una respuesta de tipo parasimpático, con el aumento de los parámetros rMSSD y HF cuando los sujetos están sometidos a una atmósfera de aire enrarecido. En este caso, compuesto por 13% de O_2 y 2,5% de CO_2. En aire enrarecido, como el de la sima estudiada, los sujetos presentaban el triple de fenómenos arrítmicos que cuando realizan una tarea con carga similar en aire estándar.

Introduction

In June 2013, a group of cavers discovered a new cavern in the Garraf Massif (Catalonia, Spain). The exploration was very difficult as it was done in air that was oxygen-poor (as low as 11.3%) and high in CO_2, at 2.5% (25,000 ppmv). The only known characteristics of this air are that it is supersaturated with H_2O and has a pH of 6.

Without any additional respiratory support, they explore to a depth of −193 m and stay for more than 2 h in an oxygen concentration of 15%.

This phenomenon of rarefied air, known for over 100 years and called *guilla* locally, occurs naturally in caves in Mallorca, Catalonia, French Roussillon and also in other parts of the world[1-4].

We can find a similar situation in mining accidents[a], submarines and other contraptions with confined or artificially maintained atmospheres[2b].

Several studies have been published regarding human tolerance to rarefied atmospheres[5-10] but there is little research describing human physiological adaptation, furthermore when there is low altitude and normal gravity.

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[a] http://es.wikipedia.org/wiki/Desastre_minero_de_Pasta_de_Conchos

[b] http://www.elperiodico.com/es/cartas/entre-todos/miedo-ascensor-del-metro-barcelona/113855.shtml

After the discovery of this cavern a study was begun to determine the different aspects of adaptation to this environment. Prior to this research we published a study[11] from which some initial recommendations for exploring the caves in the Garraf Massif[12] were developed.

The objective of this work is to describe cardiac, vascular and nervous system adaptation[13,14] during exposure to rarefied air. Due to the difficulty of having capable personnel available underground all the time to assess the risk, ensure the proper implementation of data collection protocols and move around smoothly on ropes in such an adverse situation, the collection of blood samples and analysis of exhaled gases by spirometry had to be forgone.

Ethics Committee

This study was approved by the Catalan Sports Administration ethics committee and authorized by the Garraf and Olèrdola Natural Park management.

An emergency plan with prior notification by 112 phone was drawn up with the emergency medical services of Catalonia, police and fire brigade of the government of Catalonia.

In situ respiratory support and rapid evacuation preventative measures, where necessary, were also referred to in the emergency plan.

Material and method

Twenty five subjects[c] (including 4 women) were included in the study, age: 47.1 ± 10.1; weight: 71.9 ± 10.0; height: 173.3 ± 7.1; BMI: 24.2 ± 2.6. In data relating to heart rate variability, n = 24. Prior to the study they passed a medical check-up including: background study, examination of organs and systems, spirometry, resting electrocardiogram, blood pressure measurement following international guidelines; cardiac ultrasound; clinical stress test including electrocardiogram and cardiovascular suitability study in accordance with the Miyai algorithm. The medical review was carried out in the Sports Medicine Centre under the Sports Medicine Unit of the Catalan Sports Council (Generalitat of Catalonia).

Devices used in field work

- Holter HT 103. 3-channel Holter recorder system, Eccosur®, Argentina.
- Omron model M3 automatic tensiometer.
- Oxypalm Pulse Oximeter, KTMED Inc., Medical Systems®, Korea.
- Gas analyzer for oxygen and CO_2, loaned by the General Secretariat for Sport: Multiple Gas detector (MultiRAE-IR, Rae Systems Inc., San Jose, California, USA).

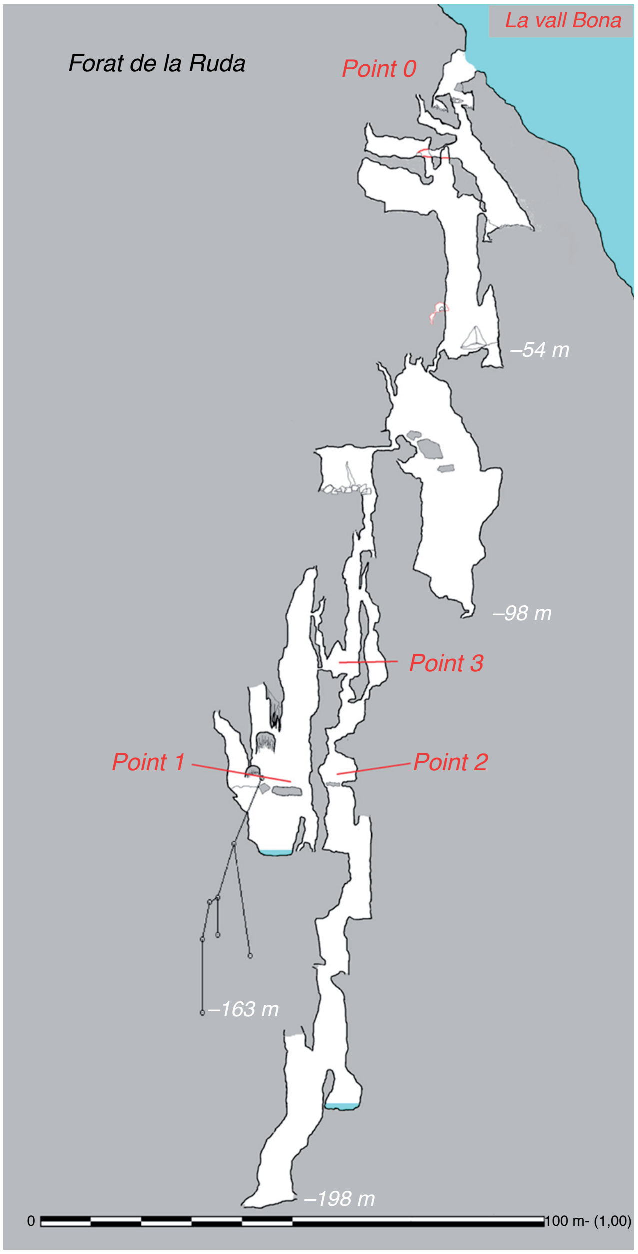

Figure 1 Topography of Forat de la Ruda pothole.
Point 1: Palermo-Buenos Aires Hall (−124 m). Point 2: Aconcagua Hall (−125 m); Point 3: La Banyera (−107 m).
Topography: Ignasi de Yzaguirre i Maura.

Features of the terrain where the study is conducted

The study is carried out in a pothole in the Garraf Massif *(Forat de la Ruda)* nearly 200 m deep (Fig. 1) in which there is rarefied air (reaching 11.3% oxygen and 25,000 ppmv CO_2). It is carried out over several descents into the cavern on successive Fridays. The volunteer group is a maximum of 2 subjects each time.

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[c] Forty two subjects do the prior check-up but, for different kinds of problems, the field tests are only done by 28 subjects, 3 of whom do not provide analysable data.

Table 1 Subjects' length of stay in rarefied air

Time below 18% oxygen		Time below 15% oxygen	
Mean	SD	Mean	SD
2 h 31 min	45 min	57 min	36 min

SD: standard deviation.

- *Test 1* (point 0, Fig. 1), normal air composition (NA). At the entrance to the *Forat de la Ruda* pothole with a temperature of 24.6 ± 5.3 °C and humidity of 52.07 ± 17.1%. Atmospheric oxygen: 20.9%; atmospheric CO_2: 0.065 ± 0.007%. It is situated at an altitude of 345 m above sea level (masl).
- *Test 2* (point 1, Fig. 1), rarefied air of natural origin (RA). *Palermo-Buenos Aires Hall*, 124 m deep in relation to the entrance; with a temperature of 16.1 ± 0.87 °C, 100% humidity and confined air H_2O with a pH = 6[d]. Atmospheric oxygen: 13.38 ± 1.5%; CO_2: 2.23 ± 0.31%, and other gases.

Technical characteristics of the subjects

Group made up of cavers, police underground and high altitude specialists and fire-fighter mountain rescue specialists.

Difficulties to be overcome in order to access the RA scenario: it is necessary to descend 9 wells with depths between 4 and 31 m using the rappel technique. In addition, there are 3 narrow passes and a narrow, 6 m long bend to get through. It takes an hour and 30 min to get past these difficulties on the way down and 3 or 4 h to return to the surface.

Schedule

At 09.00 hrs the Holter-ECG devices are fitted on 2 volunteers (in the vehicle parking lot). At 10.30 the NA test is performed. Between 12.30 and 13.30 the RA test is performed.

The NA and RA tests consist of being at physical and mental rest for 10 min, comfortably seated and listening to a sung mantra (Vijaya Devi Mantra)[e] in a breathing meditation posture[f].

The Holter monitor continues recording. After 10 min, both blood pressure and SaO_2Hb are checked.

The individuals remain on average 2 h 31 min ± 45 min below 18% oxygen and 57 ± 36 min below 15% oxygen (Table 1).

Statistics

Means and standard deviations were computed from the parameters obtained. The differences were demonstrated by means of Student's t-test for paired data. The Pearson correlation coefficient was used to assess the degree of dependence of some variables in relation to atmospheric changes. To calculate the variations in blood pressure, their modifications were expressed in standard scores (SS) and also in modifications of the percentiles they occupy in the algorithm proposed by Miyai[15] to calculate blood pressure based on HRR percentage, for the purpose of evaluating variations in heart rate (HR) despite not showing significant differences in the 2 situations studied.

In relation to heart rate variability, the parameters studied are: a) for central trend measurements derived from the standard deviation: SDNN; SDANN; SDNNindex; RMSSD; NN50; pNN50; b) references from the Fourier fast integration analysis of the r-r period sequences: TP, VLF, LF, HF and LF1/HF1. It must be said that 2 Holter ECG periods of 10 min are compared. For the assessment of arrhythmic phenomena, 2 Holter periods of 60 min including both NA and RA tests are compared.

Results

Heart rate

No statistical differences are confirmed in heart rate (HR), as evaluated by the heart recorder (Holter) over 24 h, between the NA test and the RA test during the standardized 10 min rest period (NA: 81.9 ± 15.1 bpm vs. RA: 83.8 ± 17.3 bpm; $P \leq 0.43$; NS). The NA test is preceded by a 45 min walk with all the equipment in a backpack and the RA test adds the previous activity to the task of descending quietly into the cavern (9 descent stages by rope, a climb and 4 narrow passes to get through).

Blood pressure

The subjects' blood pressure adapts to the rarefied atmosphere situation with a statistically significant increase in systolic blood pressure (NA: 130.3 ± 17.2 vs. RA: 140.2 ± 21.3 mmHg; $P \leq .0003$) and diastolic blood pressure (NA: 78.2 ± 11.0 vs. RA: 85.5 ± 11.2; $P \leq .0002$).

The pulse pressure (PAS-PAD) shows no statistically significant differences in the 2 situations studied (NA: 52.4 ± 12.9 vs. RA: 55.5 ± 14.2, without statistical significance).

The average blood pressure is NA: 94.4 ± 11.8 mmHg vs. RA: 101.6 ± 12.7 mmHg; $P < .00008$.

Heart rate variability

a) For central trend measurements derived from the standard deviation of the *r-r* periods, we analyze the data shown in Table 2.
b) For Fourier fast integration analysis of the *r-r* period sequences, see Table 3.

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[d] There may have been overhumidity and water may have formed part of the atmospheric gases breathed.

[e] By Sarva-Antah (Google Play . eMusic) http://www.youtube.com/watch?v=ghIStIQXqgw

[f] The breathing meditation technique has a parasympathetic effect. Its use in this case aims to strictly standardize the test and enable comparison. As will be seen in the results, the impact of rarefied air also has a parasympathetic effect. Do not confuse action and consequence.

Table 2 Comparison of heart rate variability between the 2 studied situations (1)

	Test 1 (NA)	Test 2 (RA)	p
SDNN	51.6 ± 15.7	62.0 ± 23.8	< 0.03
SDANN	19.6 ± 14.5	31.2 ± 20.0	< 0.04
SDNNindex	47.4 ± 17.9	56.4 ± 22.5	< 0.03
rMSSD	25.9 ± 13.8	36.9 ± 17.8	< 0.002
NN50	49.0 ± 66.2	111.7 ± 102.8	< 0.003
pNN50	7.5 ± 11.3%	15.9 ± 15.8%	< 0.01

The SDNN, SDANN, SDNNindex and rMSSD parameters are expressed in milliseconds. NN50 is expressed in number of beats. NN50: number of intervals with difference greater than 50 ms; rMSSD: the root mean square differences of successive R-R intervals; SDANN: standard deviation of the averages of NN (normal sinus to normal sinus) intervals in all 5-minute segments; SDNN: standard deviation of normal to normal R-R intervals; SDNNindex: the mean of the 5-minute standard deviations of NN intervals.

Arrhythmias

At rest, when we compare the rhythmic behaviour of the heart in normal conditions (NA) versus rarefied air (RA) and by analyzing the set of subjects, we observe a slight increase in arrhythmic events in the rarefied air situation.

We also analyze and compare performance of the 60 min that include NA (H_{NA}) versus the 60 minutes in rarefied air that include RA (H_{RA}) and the differences are significant when we analyze the set of arrhythmic events (Table 4).

- Ventricular ectopic beats (VEB), comparison of the 2 situations studied: H_{NA} = 4.6 ± 11.7 vs. H_{RA} = 13.7 ± 31.6; n = 24; *P* < = .06; NS.
- Total ectopic beats (ETB) (ventricular + supraventricular), comparison of the 2 situations studied: H_{NA} = 7.4 ± 12.9 vs. H_{RA} = 23.6 ± 47.4; n = 24; *P* < .046.

Table 3 Comparison of heart rate variability between the 2 studied situations

Fourier	Test 1 (NA)	Test 2 (RA)	p
TP	1,755.9 ± 1.324.5	2.611.5 ± 1.926.9	< 0.04
VLF	781.1 ± 513.6	1.124.8 ± 882.0	NS
LF	757.3 ± 600.0	832.8 ± 819.4	NS
HF	301.5 ± 329.4	662.3 ± 762.8	0.02
LF/HF	5.1 ± 7.3	2.3 ± 1.9	NS

Units in milliseconds squared.
HF: high frequency from 0.15 to 0.4 Hz; LF: low frequency from 0.04 to 0.15 Hz; TP: Total Power; VLF: the very low frequency from 0.0033 to 0.04 Hz.

Table 4 Number of arrhythmic phenomena comparing subjects' cardiac response in rarefied air vs. normal air

10 min strict rest			
	No. cases RA	No. cases NA	p <
Ventricular extrasystoles	30	33	NS
Supraventricular extrasystoles	20	2	NS
Total extrasystoles	50	35	NS
FC	81.5 ± 16.1	82.9 ± 16.3	NS
60 min of activity including 10 min strict rest			
	No. cases 60_{RA}	No. cases 60_{NA}	p <
Ventricular extrasystoles	328	110	.06
Supraventricular extrasystoles	238	67	NS
Total extrasystoles	566	177	.046
HR	92.0 ± 14.7	93.6 ± 17.6	NS

HR: heart rate; NA: normal air; RA: rarefied air.
60_{NA}: 60 min activity including NA period.
60_{RA}: 60 min activity including RA period.

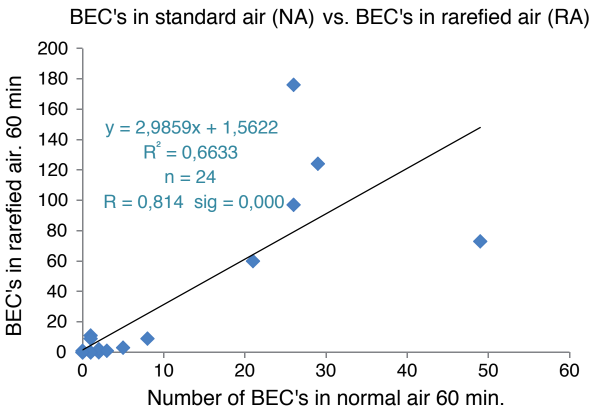

Figure 2 Correlation between ectopic beats in normal air and beats that feature in rarefied air. Comparison between periods of 60 min.

An increase in the arrhythmic events is detected when we compare the hour of air of normal proportions included in test 1 (H_{NA}) with the hour of rarefied air included in test 2 (H_{RA}). There is a clear statistical correlation between the two situations: y = 2.9859x + 1.5622; n = 24; R = .814; *P* < .0001 (Fig. 2).

Discussion

1. The results of our study show that rarefied air in the confined environment of potholes is a different setting from

a physiological point of view compared to acute exposure at high altitude (high mountain)[16-18] because, in our case, heart rate at rest tends not to rise and heart rate variability shows a parasympathetic dominance. The same occurs when we compare it to the rarefied air environment in the microgravity found in spacecraft[19]. And it is also different to the environment found in popular training tents as CO_2 levels of respiratory origin are less extreme than those in potholes.

2. Differences and similarities to previous studies: there are no known previous studies in naturally occurring confined environments like the many potholes in the Mediterranean area. Mention should be made of our own study done with volunteers in a reconstructed environment in a training tent[11] and also of the workplace study done with a mannequin in a simulation chamber[20].
3. In view of the above, the current study's inputs will complement observations made in our previous studies, such as those relating to the increased use of anaerobic metabolism resources in moderately rarefied air exercises at normal barometric pressure[21] or the clinical symptomatology, laboratory-created, in a confined rarefied air environment. Our study has confirmed the real life observations referring to blood pressure and arrhythmic phenomena that were noted in the laboratory study[11].
4. Clinical implications: our study more accurately recounts the risks to subjects who venture into confined spaces and also provides guidance on how to deal with exposure to such an environment. Being at rest will, among other things, be a useful resource for people involuntarily subjected to rarefied atmosphere who cannot leave the situation momentarily.
5. Limitations: Due to the nature of the study and the inherent risk in such an extreme atmosphere, the number of volunteers had to be limited.

Exposure to rarefied air is not common in the human species. The typical composition is 21% oxygen and 79% nitrogen plus some tiny amounts of rare gases. Carbon dioxide, at 0.06% of total volume, is part of the latter group, despite its atmospheric presence increasing significantly, linked to the use of fossil fuels:[g]

$$C_xH_y + (x + y/4)O_2 \rightarrow xCO_2 + (y/2)H_2O$$

The correlation coefficient is low when we compare changes in the diastolic blood pressure with environmental CO_2 ($r = 0.21$; $P < .04$) and higher with oxygen ($r = 0.62$; $P < .001$). The analysis of coefficients for oxygen and CO_2 shows a significance of 0.018 for oxygen and $P < .132$ for CO_2, so that the hypertensive response can be attributed to the decrease in environmental oxygen.

On the other hand, changes in heart rate variability may be due to the change in tidal volume that occurs in rarefied air (hyperventilation response) and its influence on the stretching of the right atrium-sinus node[22], though this point is insufficiently clarified. There are no changes in average heart rate (they are not significant) in the 2 situations studied but there are differences in the parameters derived from the standard deviation.

The resting heart rate[23] — historically a highly valued parameter for assessing fatigue and proper assimilation of training — is the data on which all heart rate variability studies are based in the different modalities. Its increase is characteristic of the sympathetic response. In the present case there is no significant increase in heart rate despite the dramatic change in atmospheric gaseous parameters. All of which would be consistent with parasympathetic dominance in the adaptive response. Given that none of the volunteers normally does breathing meditation, one cannot expect the induced rest to interfere with the parasympathetic response that occurs in rarefied air compared to normal air[24].

Among the other parameters used to study heart rate variability, remember that: at altitude, total spectral power (TP) tends to decrease, unlike our confined environment in which said parameter increases (1,755.9 ± 1,324.5 vs. 2,611.5 ± 1,926.9; $P < .04$)[16]. LF: 50% is due to friendly changes and its changes are difficult to interpret. HF: 90% is due to parasympathetic changes and 10% to friendly change so its variation is much easier to interpret[25]. As can be seen in our study, there are statistically significant differences in the high frequency range (HF: 301.5 vs. 662.3 ms^2; $P \leq .02$) which in this case is indicative of parasympathetic dominance.

Heart rate variability (HRV) increases and the values of the parameters relating to the parasympathetic system activity (RMSSD and HF) increase, indicating psychophysical adaptability to the situation studied[26].

The parasympathetic dominance can be interpreted as the adoption of the most profitable (automatic) economy management systems in a crisis situation of rarefied air in a confined space. On the subject of the impact of rarefied air, we note the following: all SD parameters increase in the RA situation, without an increase in the average r-r spaces. If we subject it to classic Tijvinski and Aullik[27] analysis, parasympathetic dominance has a clear significance.

In the light of the results, we see that the physiological response in a confined environment is different than at altitude[28]. In the case of the normobaric hypoxic confined atmosphere, cardiac adaptation is characterized by no increase in heart rate and a significant increase in both systolic and diastolic blood pressure and mean arterial pressure.

We agree with DiPasquale et al.[29] who, in a recent study, suggest that under hypobaria, hypoxia exerts its own effect on recovery heart rate. Therefore, normobaric hypoxia and hypobaric hypoxia might not be interchangeable environments.

Arrhythmogenic phenomena

The analysis of the relative risk (odss-ratio [OD]) of suffering arrhythmic events when we submit a subject to rarefied air in the proportions of our study (O_2: 13.38 ± 1.5% and CO_2: 2.23 ± 0.31% and other gases) is 3.2 times higher (OR:

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[g] http://ca.wikipedia.org/wiki/Combusti%C3%B3

3.2630; CI 95%: 2.7557 to 3.8637; z statistic: 13.719; significance level: $P < .0001$).

Blood pressure

The results relating to blood pressure in NA and RA are comparable and assessable. There are no differences in average heart rate in the 10 min studied (NA: 81.9 ± 15.1 bpm vs. RA: 83.6 ± 17.3 bpm; P = NS) so we can say that adaptation to rarefied air causes an increase in blood pressure without increasing heart rate.

Conclusions

1. After the standardized 10 min rest in rarefied air (RA), there is an increase in systolic, diastolic and mean arterial blood pressure.
2. The heart rate variability study, in standardized rest situation, shows a parasympathetic type of response with increased RMSSD and HF parameters when subjects are subjected to a rarefied air atmosphere (RA), composed of 13% oxygen with 2.5% CO_2.
3. In rarefied air like that of the studied pothole, the subjects presented three times as many arrhythmic phenomena than when they carried out the same tasks in non hypoxic areas with similar workloads.

Funding

The study has received the grant from the Catalan Sports Council's subvention program for carrying out research work in the field of sport sciences for the year 2014 (DOGC 6531, 31 Dec. 2013).

Conflict of interest

The authors declare they have no conflict of interest.

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