Artículo original

Heart rate variability to assess the effect of sleep deprivation in mountain troops of the chilean army: a pilot study

Claudio Nieto-Jiménez[1], **José Naranjo Orellana**[2]

[1]Centro de Lecciones Aprendidas, División Doctrina, Ejército de Chile. [2]Universidad Pablo de Olavide. Sevilla.

Recibido: 12.11.2018
Aceptado: 08.03.2019

Summary

Background: Our objective was to identify the effect of sleep deprivation on a stress test simulating a military march, via changes in heart rate variability (HRV) in special mountain troops.
Eight subjects from special mountain troops carried out a simulated march test on a treadmill. The incremental march test had 7 stages of 3 minute duration at a constant velocity of 5 km/h and slopes of 1, 3, 5, 7, 8, 9 and 10 %. To assess the HRV, two heartbeat records were taken over 5 minutes in dorsal decubitus position before and after the march test; the first session took place without sleep deprivation, and the following day with sleep deprivation.
Results: The main finding of this study is that the physiological stress imposed by the simulated treadmill march is the same with and without sleep deprivation.
There were no significant differences between pre and post HRV data in any of the situations, but effect size was moderate or large (d=0.2 was considered as the Smallest Worthwhile Change). indicating a highly relevant response. However, after comparing with and without sleep deprivation tests no changes were found (non-significant and non-relevant).
Conclusions: The stress test performed, did not present differences in physical and physiological responses while being deprived of sleep over 24 hours.
A simple test is proposed to evaluate the effect of sleep deprivation as a stressor agent. A treadmill test at a constant speed with increasing slopes would be performed and repeated the following day after 24 hours of sleep deprivation.

Key words:
Heart rate variability.
Sleep deprivation.
Special Mountain Troops.

Variabilidad de la frecuencia cardíaca para evaluar el efecto de la privación del sueño en tropas de montaña del ejército chileno: un estudio piloto

Resumen

Introducción: Nuestro objetivo fue identificar el efecto de la falta de sueño en una prueba de esfuerzo que simula una marcha militar, a través de cambios en la variabilidad de la frecuencia cardiaca (VFC) en tropas especiales de montaña.
Ocho sujetos de tropas especiales de montaña realizaron una prueba de marcha simulada en una cinta de correr. La prueba de marcha incremental tuvo 7 etapas de 3 minutos de duración a una velocidad constante de 5 km/h y pendientes de 1, 3, 5, 7, 8, 9 y 10%. Para evaluar la VFC, se tomaron los registros de latidos latido del corazón durante 5 minutos en posición de decúbito dorsal antes y después de la prueba de marcha; la primera sesión tuvo lugar sin privación de sueño y al día siguiente con privación de sueño.
Resultados: El principal hallazgo de este estudio es que el estrés fisiológico impuesto por la marcha simulada de la cinta rodante es el mismo con y sin privación del sueño.
No hubo diferencias significativas entre los datos de VFC anteriores y posteriores en ninguna de las situaciones, pero el tamaño del efecto fue moderado o grande (d = 0.2 se consideró como umbral de cambio pequeño). Indica una respuesta altamente relevante. Sin embargo, después de comparar con y sin las pruebas de privación de sueño, no se encontraron cambios (no significativos y no relevantes).
Conclusiones: La prueba de esfuerzo realizada no presentó diferencias en las respuestas físicas y fisiológicas al estar privada de sueño durante 24 horas.
Se propone una prueba simple para evaluar el efecto de la falta de sueño como agente estresante. Se realizaría una prueba de la cinta rodante a una velocidad constante con pendientes crecientes y se repetiría al día siguiente después de 24 horas de falta de sueño.

Palabras clave:
Variabilidad de la frecuencia cardiaca.
Privación de sueño.
Tropas Especiales de Montaña.

Correspondencia: Claudio Nieto-Jiménez
E-mail: c.nieto@udd.cl

Introduction

The completion of a mission on the battlefield is the result of the sum of multiple factors, in their preparation; soldiers must be conditioned to resist fatigue, fear and doubt, all of which are characteristic of the human condition. This requires highly prepared subjects in good physical conditions to allow for optimum performance under stressful situations.

One of the main components of that plan is to improve the morphological, fitness and physiological profile as well as basic and specific military skills[1].

Sleep deprivation is one of the main stressor agents in the training of soldiers, particularly in mountain special forces of Chilean Army[2]. We know that the lack of sleep affects directly to the physical status and the capacity to perform specific tasks in soldiers[2,3].

It has been observed that a single night of sleep deprivation may affect the resistance performance of a 30 minute treadmill run at an intensity of 60% of the VO_2max and alter cardio-respiratory, thermoregulatory and perceptual responses to exercise[4]. A study undertaken by the Croatian Army[3] for Special Operations, reported the influence of basic training on specific shooting tasks under sleep deprivation conditions. The results showed that basic training had a positive impact on the reduction of the effects of sleep deprivation in shooting related tasks. The data obtained suggests that during basic training (62 days) there was an adaptation to stress as well as an improvement in weapons handling skills, which contributes significantly to improved shooting results in stressful conditions, mainly in terms of sleep deprivation.

Likewise, Tyyskä[5] *et al.* (2010) investigated the links between physical fitness, sleep duration and hormonal responses during military training over 15 days while carrying out offensive maneuvers in a rural area. On average, the subjects slept 6.20 hours per day, but their sleeping patterns were altered due to guard shifts. The study found hormonal changes related to a lack of sleep and low physical fitness.

Ricardo[6] *et al*. (2009) determined that 30 hour of sleep deprivation did not alter leukocyte traffic, neutrophil degranulation or resting S-IGa responses.

But in addition to affecting the general physical state of the subject, sleep deprivation should have some kind of impact on the balance of the sympathetic-parasympathetic system, especially when it is required for some specific task[7].

Heart rate variability (HRV) is a non-invasive tool to analyze changes in the autonomic nervous system (ANS)[8-10] and it is used to assess adaptations to effort in different circumstances[11-14].

A study carried out on soldiers Huovinen[15] *et al*. reported changes in some indicators of HRV with a positive correlation with changes in testosterone and cortisol. However, we have not found any study utilizing HRV to evaluate the effect of sleep deprivation in the execution of military tasks. On the other hand, there is no simple test to evaluate the effect of sleep deprivation on the physical performance in Special Forces troops.

The aim of this pilot study was to identify the effect of sleep deprivation on HRV during a effort test (simulating a military march) in special mountain troops in the Chilean Army; and to propose it as a pilot simple test to evaluate the role of sleep deprivation as a stressor agent in this population.

Material and method

Eight subjects from special mountain troops carried out a simulated march test on a treadmill, in full combat equipment. They spent one night without sleep during a planning exercise in a classroom and returned to carry out the test the following day. The evaluated soldiers belonged to a Special Forces patrol with five years of experience working together in winter and summer mountain training. All were volunteers; they were informed of the procedures and consequently signed a consent form. The study had the approval of the Ethics Committee of Health Sciences of Santiago´s Military Hospital and was carried out in accordance with the dispositions of the Helsinki Declaration[16].

An incremental march test was carried out on a treadmill with 16.5 kg of weight in individual combat equipment. The test had 7 stages of 3 minute durations and slopes of 1, 3, 5, 7, 8, 9 and 10% as well as a constant velocity of 5 km/h. To assess the HRV, a heartbeat record was taken over 5 minutes in dorsal decubitus position prior to the march test (Pre) and another upon completion (Post); the first session took place without sleep deprivation, and the following day, at the same hour (06:00 a.m.), the procedure was repeated after a night of sleep deprivation.

Prior to the tests, the weight was measured with a Tanita weighing machine (Tanita Ironman BC1500, Japan, 2015). All subjects wore a heart rate monitor Polar V800 (Polar, Kempele, Finland). The data from this device were downloaded via USB throw the application Polar FlowSync in order to obtain a time series of the RR intervals (beat by beat). This time series was analyzed with the software Kubios HRV[17] (University of Eastern Finland, Kuopio, Finland).

The general variables obtained from the effort test were: resting heart rate (rHR), prior to the test; theoretical maximum heart rate (HRmax); exercising heart rate (eHR) for each stage of the test; the relative intensity (%) for every step obtained via the Karvonen[18] equation and the total power (Watts) calculated from the velocity, gradient and body mass with equipment.

The use of slopes requires to have into account the vertical component of velocity in the calculation of the work and total power generated on a treadmill. The most common way to take this into account is via the sine of the angle α formed by the treadmill and the horizontal[19] or by substituting the sine α by the percentage of slope of the treadmill, divided by 100[20], given that for very small values of α, the numerical value of sine α is very close to that of the slope expressed in decimals, so the following equation may be used[21]:

$$P = m * g * v * p * 0{,}278$$

Where v is the velocity expressed in km/h; g is the average acceleration of gravity (9,8 m/s2); m is the subject mass in kg and p is the percentage of gradient of the treadmill, divided by 100.

The HRV variables used for the analysis in the time domine[22,23] were: RR; time interval between two R waves (ms); SDNN: standard deviation of the RR; RMSSD: square root of the average of the differences of the sum of the squares between adjacent RR intervals (ms); pNN50: percentage of adjacent RR intervals which differ more than 50 ms (%);

The transversal axis (SD1) and the longitudinal axis (SD2) were determined in the Poincaré´s plot[23] and, in accordance with Naranjo[24] *et al.*, the Stress Score (SS) was calculated as the inverse of the SD2 multiplied by 1000 and the sympathetic-parasympathetic ratio (R-S/Ps) as the ratio between the SS and SD1. For analysis purposes of the autonomic balance, the Napierian logarithm of the SS was used (LnSS) as an indicator of sympathetic activity and the LnRMSSD as an indicator of parasympathetic activity.

Statistical Analysis

A descriptive study was carried out, presenting the data as averages, standard deviations (SD) and variation coefficient (VC).

For hypothesis contrasting, the normality of distributions was tested using the SHAPIRO-WILK test, and the LEVENE test was used to establish the equality of variances.

For the HRV data analysis a multiple comparison ANOVA test was used for the 4 distributions (pre and post without sleep deprivation and pre and post with sleep deprivation) utilizing BONFERRONI´s *post-hoc* test.

For the analysis of general variables of both tests (rHR, HRmax, eHR, intensity and total power) a *t*-Studen test was used for paired samples.

In all cases the significance level was fixed at $p<0,05$.

Given the reduced sample size, significant results were not expected to be achieved with conventional statistical hypothesis contrast; consequently, in order to assess the changes between the different variables the effect size (ES) was calculated throw the Cohen's d[25] using the intervals proposed by Hopkins[26]: <0.2 = trivial, 0.20-0.59 = small; 0.6-1.2 = moderate; ≥1.2 = large.

Results

Table 1 shows the average and standard deviatios (SD) for age, weight (kg), theoretical HRmax, rHR, maximal eHR and maximal intensity of the test. The values of p comparing rHR, eHR and intensity between the situation 1 (no sleep deprivation) and the situation 2 (sleep deprivation) indicate that changes were not statistically significant. The values of d for both situations show that the effect size was trivial or small.

Table 2 shows general data for every stage in the effort test (power, speed and slope) together with average, SD and VC of eHR in both situations: with and without sleep deprivation. No significant differences were observed between both conditions.

Figure 1 shows the evolution of the eHR in relation to the power of each stage in both tests.

Table 3 shows pre and post results of HRV with and without sleep deprivation. The p values for PRE-POST comparisons were all above 0.8; the values of d are shown in the table and we can see that the effect size is medium or large for all the variables.

When the HRV values post-test are compared in both situations, there are no significant differences (p>0,5 in all the cases) and the effect size is small for all the variables (d<0,2).

Figures 2 shows the changes in LnSS (A) and LnRMSSD (B) in both tests as indicators of sympathetic and parasympathetic activity respectively.

Figure 1. Exercising Heart Rate (eHR) data in relation to the power of each stage.

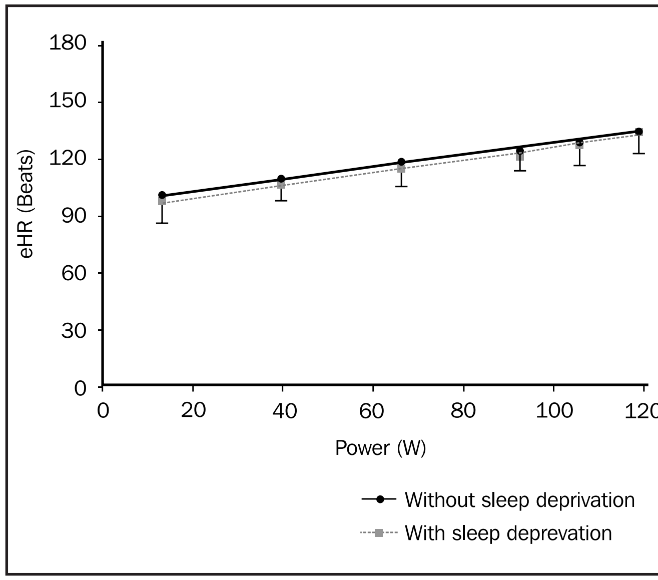


Table 1.

Subject	Age (years)	Weight (kg)	Theoretical HRmax	rHR 1	rHR 2	Maximal eHR 1	Maximal eHR 2	Maximal Intensity 1	Maximal Intensity 2
1	32	80.3	188	48	44	138	133	0.64	0.62
2	33	76.9	187	53	52	150	143	0.72	0.67
3	31	78.1	189	51	52	130	126	0.57	0.54
4	31	80.7	189	40	40	132	128	0.62	0.59
5	27	71.7	193	84	52	140	130	0.51	0.55
6	24	81.2	196	56	52	125	127	0.49	0.52
7	28	83.7	192	64	61	166	162	0.80	0.77
8	22	91.5	198	49	50	145	146	0.64	0.65
Average	28.50	80.51	191.50	55.69	50.45	140.75	136.88	0.63	0.61
SD	3.96	5.70	3.96	13.40	6.36	13.04	12.59	0.10	0.08
				p =0.33		p =0.55		p =0.86	
				d =0.53		d =0.30		d =0.12	

The measurements without sleep deprivation are identified with (1) and the measurements with sleep deprivation with (2). The values p and d correspond to the comparison between scenarios 1 and 2. (d <0.2= trivial, 0.20-0.59= small; 0.6-1.2= moderate; >1.2= large). rHR: Resting HR; eHR: Excercising HR.

Table 2.

				eHR (beats)					
				Without sleep deprivation			With sleep deprivation		
Stage	Power (Watt)	Speed (km/h)	Slope (%)	Average	SD	VC	Average	SD	VC
1	13.21	5	1	100.125	14.623	15%	96.75	11.498	12%
2	39.64	5	3	108.75	10.195	9%	105.25	8.225	8%
3	66.07	5	5	116.5	12.107	10%	114.125	9.833	9%
4	92.49	5	7	122.5	9.827	8%	119.875	7.200	6%
5	105.71	5	8	127.5	11.352	9%	126.25	10.512	8%
6	118.92	5	9	133.75	12.116	9%	133	11.288	8%
7	132.13	5	10	140.75	13.036	9%	136.875	12.586	9%

Exercising heart rate (eHR) data corresponding to the stages of the test. (SD: Standard Deviation; VC: Variation coefficient).

Table 3.

		Without sleep deprivation			With sleep deprivation		
		PRE	POST	d	PRE	POST	d
RR	Average	1124.50	825.00	1.49	1207.59	870.81	1.99
	SD	231.31	172.02		160.03	178.59	
	VC	0.21	0.21		0.13	0.21	
SD NN	Average	88.08	57.15	0.99	109.77	58.75	1,99
	SD	29.51	32.98		25.52	25.71	
	VC	0.34	0.58		0.23	0.44	
RMSSD	Average	80.69	31.86	1.44	92.85	35.73	2.09
	SD	38.20	29.78		25.63	28.93	
	VC	0.47	0.93		0.28	0-81	
LnRMSSD	Average	4.26	3.03	1.48	4.49	3-24	2.02
	SD	0.61	1.05		0.30	0-95	
	VC	0.14	0.34		0.07	0.29	
pNN50	Average	44.38	16.49	1.29	54.36	14.31	2.62
	SD	21.45	21.65		10.24	20.38	
	VC	0.48	1.31		0.19	1.42	
SD1	Average	57.23	25.47	1.18	65.79	25.25	2.09
	SD	27.05	26.66		18.16	20.61	
	VC	0.47	1.05		0.28	0.82	
SD2	Average	109.26	75.71	0.86	139.46	78.48	1.80
	SD	37.77	40.07		35.89	31.78	
	VC	0.35	0,53		0.26	0.40	
SS	Average	10.22	16.33	1.10	7.61	14.46	1.88
	SD	3.63	7.48		2.04	5-26	
	VC	0.36	0.46		0.27	0,36	
LnSS	Average	2.27	2.69	0.99	2.00	2.61	1.90
	SD	0.36	0.50		0.26	0.38	
	VC	0.16	0.18		0.13	0,15	
Ratio	Average	0.28	2.45	1.10	0.13	1.56	1.31
	SD	0.31	3.65		0.06	2.14	
	VC	1.11	1.49		0.43	1.37	

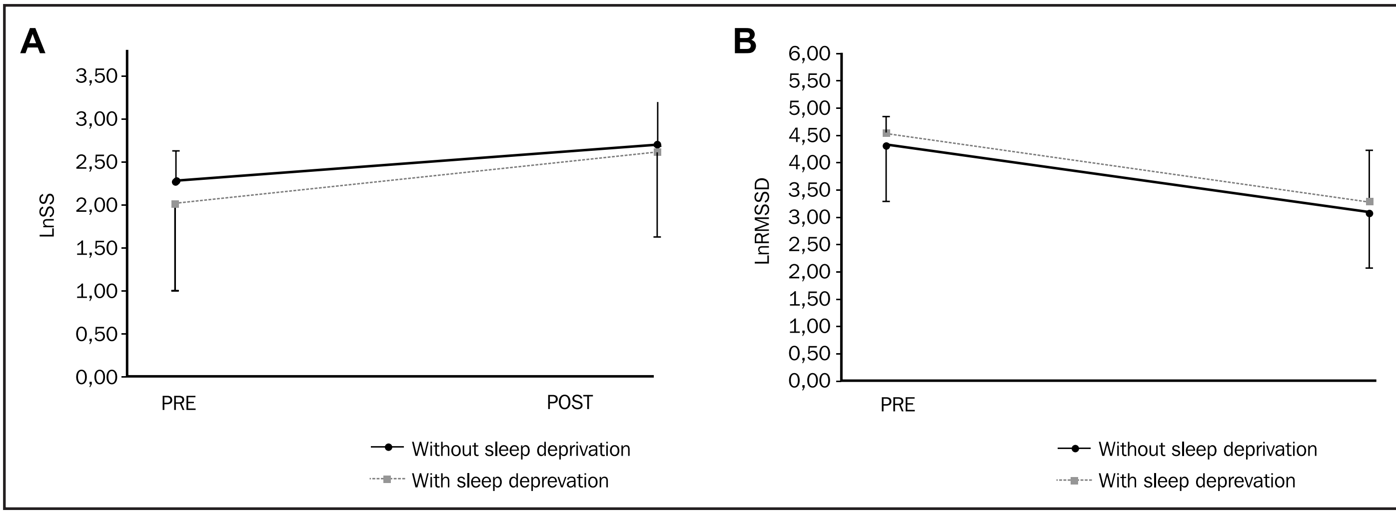
RR: RR interval (ms). SDNN: Standard deviation of the RR intervals. RMSSD: square root of the average of the differences of the sum of the squares between adjacent RR intervals (ms). LnrMSSD: Naperian logarithm of the RMSSD. pNN50: number of adjacent pairs in the RR interval which differ more than 50 ms divided by the total number of RR intervals (%).SD1: transversal axis of Poincare´s Plot. SD2: longitudinal axis of Poincare´s Plot. Stress Score(SS): opposite to the SD2, multiplied by 1000. LnSS: Naperian logarithm of the SS. R-S/Ps: Sympathetic-parasympathetic ratio: quotient between the SS and SD1. Effect size: (d <0.2= trivial, 0.20-0.59= small; 0.6-1.2= moderate; ≥1.2= large.)
The values of p between PRE and POST were all above 0.8. The values of d PRE-POST are shown in order to estimate size of effect. The p value between POST with and without sleep deprivation was >0,5 for all variables and the value of d was <0,2 in all the cases.

Discussion

The main finding of this study is that the physiological and physical stress induced by the simulated treadmill march in experienced and well-trained soldiers is the same with and without sleep deprivation.

We know that the sample is small (N=8) and that this would be an obstacle to the generalization of results, but given that this work is only a pilot study, we preferred to prioritize the fact that the 8 subjects are highly qualified soldiers well trained in mountain military tasks and who have been working together for five years in the same patrol of Special Forces. For this reason, in this pilot study it is very valuable for us to analyze their response to sleep deprivation, taking it as a reference to propose an evaluation test that, logically, should be validated later in different circumstances.

Figure 2. LnSS: Natural logarithm of Stress Score. LnRMSSD: Natural logarithm of the square root of the average of the difference of the sum of the squares between adjacent RR intervals (ms).



Effect Size: d <0.2= trivial, 0.20-0.59= small; 0.6-1.2= moderate; ≥1.2= large.

As indicated above, with such a small sample it was not reasonable to expect significant differences when using conventional hypothesis testing techniques. In fact, there was no significant differences between the pre and post data in any of the situations. However, the effect size was very important (d=0,99 for the LnSS without sleep deprivation; d=1,48 for Ln RMSSD without sleep deprivation; d=1,9 for the LnSS with sleep deprivation and d=2 for LnRMSSD with sleep deprivation), indicating that the changes PRE-POST were highly relevant.

Values of rHR and maximal intensity in the test are not influenced by sleep deprivation as shown by the fact that the effect size is small for the rHR (d=0,53 and trivial for the intensity (d=0,12) (Table 1). The final intensity was 63% for test 1 and 61% for test 2 (Table 1), being the same intensity used by Oliver[4].

The eHR values (Table 2) show practically identical behavior in both situations. As shown in Figure 1, they were not affected by the lack of sleep. These findings are consistent with previous studies by Martin & Haney[27] (1982).

Concerning HRV, we can see in both tests a drop of variables indicating parasympathetic activity (SDNN, RMSSD, LnRMSSD, pNN50 y SD1) and an increase in those indicating sympathetic activity (SD2, SS y LnSS), taking into account that the SD2 value is opposite to sympathetic activity (Table 3). On the other hand, the value of the ratio S:PS is normal at rest but it increases after exercise showing a sympathetic prevalence both with and without sleep deprivation (Table 3). As such, we are observing the expected response after an exercise load. Nevertheless, the question is whether or not the ANS response to this work load is different when the subjects are sleep deprived, or to put it differently, if the internal load representing this test is higher after 24 hours of sleep deprivation.

In this sense, no statistical significance is observed in the p-value for the HRV values in either of the two tests studied (with and without sleep deprivation) (Table 3), possibly due to the reduced sample size, being a pilot study. Regardless, the effect size is relevant for all the variables, especially for those used in this study for the evaluation of sympathetic and parasympathetic states: the LnSS (d=0,99 without sleep deprivation and d=1,90 with sleep deprivation) and the LnRMSSD (d=1.48 without sleep deprivation and d=2,02 with sleep deprivation) (Table 3).

The variation coefficient in our study (VC) for the LnrMSSD increases with the effort test both without sleep deprivation (14% and 34%) and with sleep deprivation (7% and 29%). (Table 3). Although Buchheit[28] observed individual daily fluctuations of this resting variable of around 10-20%, in our study the changes while resting represent inferior values, between 7% and 14%.

The VC for the LnSS, nevertheless, shows much smaller changes with the exercise, both without sleep deprivation (16% and 18%) and with sleep deprivation (13% and 15%). Although there were no references in the literature for the VC of this variable, it is found to be within the margins aforementioned by Buchheit for the LnRMSSD.

We consider that it would be highly useful to have a simple test in order to evaluate the effect of sleep deprivation as a stressor agent. Our data seems to reflect that the proposed effort test induces relevant changes to sympathetic-parasympathetic balance, but that these are exactly the same when subjects are sleep deprived. On another hand, the general test data (intensity and exercising heart rate) are the same with and without sleep deprivation.

This is at least what happens in highly trained soldiers and for that reason can be a good reference to assess the response of other subjects to this circumstance.

Based on these data, we propose to use this test as follow:

- To carry out the proposed test at a constant speed with increasing slopes, and repeat the process the following day after 24 hours of sleep deprivation.
- The exercising HR reached must not differ more than 10% in both tests (Table 2; VC=9% for eHR)
- Sympathetic stress induced by the effort test (LnSS) must be the same with and without sleep deprivation, accepting a maximum difference of +15% (Table 3; VC=15% for the LnSS).

– The decrease in parasympathetic modulation (LnRMSSD), induced by the effort test, must be the same with and without sleep deprivation, accepting a maximum difference of -30% (Table 3; VC= -29%).

The main limitation in this study could be the reduced sample size; but, as it is a pilot study, we have established as a priority the selection of subjects who are members of the same patrol in special mountains operations forces, with 5 years' experience with this type of training. All of them had previous experiences with different stressor agents and competences in extreme environments. In this manner we have guaranteed: a) that the subjects studied have important training in terms of adaptation to stressor agents (sleep deprivation included) and as such their responses may serve as a clear reference to evaluate other subjects; and b) that the sample, although small, is sufficiently homogenous in terms of fitness and training.

Conclusion

The response of HRV after a simulated march on a treadmill did not present differences in trained soldiers when they are deprived of sleep over a 24 hours period.

This simple test would be useful to evaluate the effect of sleep deprivation as a stressor agent.

Acknowledgment

Special Mountain Force of Special Operations Brigade from the Chilean Army, to Major Miguel Reyes, and área of research in physical education on combat, learning and teaching at the Doctrine Division.

Ethics Approval Committee

Santiago´s Military Hospital-HOSMIL-DIVDOC.

Conflict of interest

The authors do not declare a conflict of interest.

Bibliography

1. Sporiš G, Harasin D, Baić M, Krističević T, Krakan I, Milanović Z, *et al.* Effects of two different 5 weeks training programs on the physical fitness of military recruits. *Colleg Antropol.* 2014;38 (Supplement 2):157-64.
2. Nieto C, Cárcamo M. Training and evaluation of military physical capacity. Review of the literature. *R Española de Educ Fís y Deporte.* 2016;(415):75-86.
3. Jovanović M, Sporiš G, Šopar J, Harasin D, Matika D. The effects of basic military training on shooting tasks in conditions of sleep deprivation. *Kinesiology*. 2012;44(1):169-77.
4. Oliver SJ, Costa RJ, Laing SJ, Bilzon JL, Walsh NP. One night of sleep deprivation decreases treadmill endurance performance. *Eur J Appl Physiol* 2009;107(2):155-61.
5. Tyyskä J, Kokko J, Salonen M, Koivu M, Kyröläinen H. Association with physical fitness, serum hormones and sleep during a 15-day military field training. *J Sci Med Sport.* 2010;13(3):356-9.
6. Ricardo JC, Cartner L, Oliver SJ, Laing SJ, Walters R, Bilzon JL, *et al.* No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise. *Eur J Appl Physiol.* 2009;105(3):499-504.
7. Frank MG. The mystery of sleep function: current perspectives and future directions. *R Neur.* 2006;17(4):375-92.
8. Aubert AE, Seps B, Beckers F. Heart rate variability in athletes. Sport Med. 2003;33(12):889-919.
9. Fridén J, Lieber RL, Hargreaves M, Urhausen A. Recovery after Training–Inflammation, Metabolism, Tissue Repair and Overtraining. *Sport Med: Basic Sci Clin Asp Sport Inj Physiol Act.* 2003;189-200.
10. Cachadiña ES, de la Cruz Torres B, Orellana JN. Comparative study of the weekly profiles of creatin kinase, urea and variability of heart rate in Spanish elite rowers. *Arch. Med Deporte*. 2012;(152):952-8.
11. Impellizzeri FM, Rampinini E, Marcora SM. Physiological assessment of aerobic training in soccer. *J Sport Sci.* 2005;23(6):583-92.
12. Bärtsch P, Saltin B. General introduction to altitude adaptation and mountain sickness. *Scand J Med Sci Sport*. 2008;18(s1):1-10.
13. Henning PC, Park BS, Kim JS. Physiological decrements during sustained military operational stress. Military Med. 2011;176(9):991-7.
14. Halson SL. Monitoring training load to understand fatigue in athletes. *Sport Med.* 2014;44(2):139-47.
15. Huovinen J, Tulppo M, Nissilä J, Linnamo V, Häkkinen K, Kyrolainen H. Relationship between heart rate variability and the serum testosterone-to-cortisol ratio during military service. *Eur J of Sport Sci*. 2009;9(5):277-84.
16. WMA Declaration of Helsinki – Ethical Principles For Medical Research Involving Human Subjects. 64th WMA General Assembly, Fortaleza, Brazil, October 2013. Disponible en: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/
17. Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO, Karjalainen PA. Kubios HRV–heart rate variability analysis software. *Comp Met Prog Biome.* 2014;113(1):210-20.
18. Karvonen MJ. The effects of training on heart rate; a longitudinal study. *Ann Med Exp Biol Fenn*. 1957;35:307-15.
19. McArdle WD, Katch FI, Katch VL. *Fundamentos de Fisiología del Ejercicio*, Madrid. Ed. McGraw-Hill-Interamericana, 2004. p. 137.
20. Mora Rodríguez R. *Fisiología del deporte y el Ejercicio*, Madrid, Ed. Médica Panamericana, 2009. p. 35.
21. Naranjo Orellana J. El Laboratorio de Fisiología del Ejercicio. Instrumentación. Protocolos de pruebas de esfuerzo, En. Naranjo Orellana J, Santalla Hernández A, Manonelles Marqueta P. *Valoración funcional del deportista en el Laboratorio.* Barcelona, Esmon Publicidad; 2013. p.47.
22. Camm AJ, Malik M, Bigger JT, Breithardt G, Cerutti S, Cohen RJ, *et al.* Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. *Eur Heart J.* 1996;17(3):354-81.
23. Tulppo MP, Makikallio TH, Takala TE, Seppanen THHV, Huikuri HV. Quantitative beat-to-beat analysis of heart rate dynamics during exercise. *Ame J Physiol Heart Circul Physiol.* 1996;271(1):H244-52.
24. Naranjo J, De La Cruz B, Sarabia C, E De Hoyo Lora M, Dominguez S. Two New Indexes for the Assessment of Autonomic Balance in Elite Soccer Players. *Int J Sport Physiol Perform*. 2015;10:452-7.
25. Cohen, J. *Statistical power analysis for behavioral sciences.* Hillsdale, NJ: LEA; 1988;p.412-14.
26. Hopkins W, Marshall S, Batterham A, Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci in Sport Exerc.* 2009; 41(1), 3.
27. Martin BJ, Haney R. Self-selected exercise intensity is unchanged by sleep loss. *Eur J appl Physiol Occup Physiol.* 1982;49(1):79-86.
28. Buchheit M. Monitoring training status with HR measures: do all roads lead to Rome?. *Front Physiol.* 2014;5:73.