Hormonal and haematological effects in a low-altitude winter march on chilean military

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Received: 11.06.2018
Accepted: 17.10.2018

Summary

Introduction: Intermittent exposures at high altitude have acute effects on some biological markers, such as testosterone, but not at low altitude. Since the training of soldiers should carry out specific military activities, is very important to asses physiological changes that can occur in particular circumstances (such as altitude) but during the performance of the activities of the military units.
Objective: To identify the hematological changes and the hormones Free Testosterone (TL), Total Testosterone (TT) and Cortisol during a nocturnal march at low altitude in soldiers of mountain operations.
Methodology: 32 male military (26.3 ± 4.50 years, 75.1 ± 7.6 kg) performed a nocturnal winter march with equipment between 902 and 1648 m of altitude. Blood samples were obtained before and after the march, and TL, TT, cortisol and blood count were measured: red blood cells (Hmt), hemoglobin (Hb), hematocrit (Htto) and mean corpuscular volume (MCV).
Results: There was a significant decrease in TL and TT values without changes in plasmatic cortisol. A reduction in the values of Hmt, Hb, Htto and VCM has also been observed.
Conclusion: A winter march with combat equipment, at low altitude and with a unevenness of 746 m, produces a significant decrease in the plasma values of Testosterone (free and total) in soldiers of mountain operations. No changes in cortisol values are observed. A significant reduction of red blood cells, hemoglobin, hematocrit and MCV is detected, which could be due to a hemodilution effect.

Key words:
Cortisol. Testosterone. Mountain troops. March.

Efectos hormonales y hematológicos en una marcha invernal de baja altitud en militares chilenos

Resumen

Introducción: Las exposiciones intermitentes a gran altitud tienen efectos agudos sobre algunos marcadores biológicos, como la testosterona, pero no así en baja altitud. Dado que el entrenamiento de soldados debería ir asociado a tareas militares específicas, adquiere gran importancia valorar los cambios fisiológicos que puedan producirse en determinadas circunstancias (como la altitud) pero durante la realización de actividades propias de las unidades militares.
Objetivo: Identificar los cambios hematológicos y en las hormonas Testosterona Libre (TL), Testosterona Total (TT) y Cortisol en una marcha nocturna a baja altitud en soldados de operaciones en montaña.
Metodología: 32 Militares masculinos (26,3 ± 4,50 años, 75,1 ± 7,6 kg) realizaron una marcha invernal nocturna con equipo y un desnivel entre los 902 y 1648 m. Se obtuvieron muestras de sangre antes y después de la marcha y se midió TL, TT, cortisol y hemograma: hematíes (Hmt), hemoglobina (Hb), hematocrito (Htto) y volumen corpuscular medio (VCM).
Resultados: Se produjo un descenso significativo de los valores de TL y TT sin cambios en el cortisol plasmático. También se observó un descenso en las cifras de Hmt, Hb, Htto y VCM.
Conclusión: Una marcha invernal con equipo de combate, en baja altitud y con un desnivel de 746 m, produce un descenso significativo de los valores plasmáticos de Testosterona (libre y total) en soldados de una unidad de operaciones en montaña. No se observan cambios en los valores de cortisol. Se detecta una reducción significativa de hematíes, hemoglobina, hematocrito y VCM que podrían deberse a un efecto de hemodilución.

Palabras clave:
Cortisol. Testosterona. Tropas de montaña. Marcha.

Funds allocated to the combat research program of the Doctrine Division, the Education and Doctrine Command of the Chilean Army. 2016

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Introduction

In the Chilean Army, personnel in the mountain troops must be highly specialised and prepared, in order to offer rapid and effective responses in different scenarios under specific stressors.

One of the most common stressors for the Chilean Army in the mountain operating environment is fatigue caused by marching in combat simulated conditions, with regard to equipment and adverse weather conditions. However, occasionally, an added factor is marching at altitude conditions, with the influences that this has on the physical performance of the subjects[1]. Due to the fact that these two factors are often combined in mountain troop operations, it is difficult to differentiate the influence of each one.

In 2010 a retrospective analysis by the United States Army[2] brought to light a considerable decrease in cold-weather injuries since the Korean war (6,300 injuries) up to operations in Afghanistan (only 19), attributing this change to better knowledge of the circumstances and to improvements in the equipment of the armed forces.

On the other hand, altitude training traditionally takes place through long acclimatisation sojourns. However, given that the military operations to be performed in these environments generally have little time for preparation, it would be of interest to know how this preparation could be made more efficient. In 2014, the British Army[3] made a review of the sporting literature that dealt with this problem, in an endeavour to draw conclusions that could be applicable to military training. Given that, in all these strategies, it is necessary to combine the best physiological adaptation in the least possible time with training sessions at appropriate intensities, it is important to know the effect that both factors (altitude and intensity) have on physiological variables.

In 2007, Muza[4] conducted a review on the effects of daily intermittent hypoxic exposures to induce altitude acclimatisation, for the purpose of considering the potential utilization of this approach in military training. He concluded that exposures of at least one and a half hours are required for at least one week and at altitudes that are equal to or higher than 4,000 m and also that the effect of intermittent exposures at lower altitudes is not documented.

However, intermittent exposures at high altitudes have acute effects on some biological markers, such as testosterone[5]. Given that the training of soldiers must be associated with specific military tasks[6], it is extremely important to ensure that the assessment of the physiological changes that may occur in certain circumstances (such as altitude) is based on the performance of activities that are specific to the military units.

The problem lies in the fact no studies in these circumstances have been reported in the literature and, if we resort to sports training literature, we find some highly diverse information with regard to the blood count values of certain hormones due to some very different conditions and protocols. Thus, following exercise at high altitudes, some authors report increases in cortisol[7-9] while others find no changes[10,11].

With regard to the exposure time at high altitudes, some authors have observed that through gradual ascents, the cortisol levels at rest do not change[12], while others report that the subjects rapidly exposed to hypoxic conditions (either in a hypobaric chamber or using a vehicle or helicopters for a rapid ascent) do show increases in cortisol[9,13,14].

A similar situation occurs with testosterone at high altitudes. Some studies report a drop in testosterone in a mountain climbing training programme[15] while others find an increase in the testosterone values in situations of acute exposure to high altitudes[16].

However, isolated changes in the values of cortisol or testosterone are important, given that, in the sports field, the free testosterone to cortisol ratio has been used as an indicator of training load for some time now[17,18], being a marker of overexertion, even for soldiers subjected to extreme loads[19]. It is therefore a useful tool that makes it possible to intervene in the planning before any pathological changes occur in athletes[18,20-22].

It is important to ensure that the study of the physiological adaptations of the armed forces to altitude (or to any other variable) is associated with specific tasks. Given that no studies exist with these characteristics, the aim of this work is to analyse the changes occurring in the hormonal values and red blood cells of soldiers from the Chilean Army taking part in mountain operations during a low-altitude nighttime winter march with full combat equipment.

Material and method

Ethical aspects

This investigation was approved by the Health Science Research Ethics Committee, Military Hospital of Santiago, observing the provisions of the declaration of Helsinki.

The soldiers were informed of the procedure and agreed to voluntarily participate by signing a consent form.

Study population

Thirty-two male soldiers (aged 26.3 ± 4.50 years, weight 75.1 ± 7.6 kg) conducted a nighttime winter march in the locality of Lonquimay, Chile, with an initial altitude of 902 m and ascending to 1,648 m, carrying equipment weighing 28 kg. It took them 5 h 38 min. to go from the base camp (902 m) and climb to an altitude of 1,648 m and return by the same route. They travelled a distance of 24.2 km with an average slope of 6.5%, and a 20.1% maximum grade. The average environmental temperature during the march was 2ºC.

All the subjects had been at the base camp, located at 902 meters, for 12 weeks prior to the march.

Taking of blood samples

Two blood samples were obtained for each subject at the same time, at the start of the march (PRE) and at the return to the base camp (POST), for subsequent analysis. The PRE fasting sample was obtained at 06:00 and the subjects spent the day in the base camp classroom planning the route for the march, with no type of physical activity until the start of the march at 00:00 (midnight). The POST sample was taken on the return of the subjects to the base camp at 06:00.

All samples were obtained by the unit's military nurses through venipuncture in the forearm using the Venoject® system and following the stipulated procedure of the Clinical Laboratory of the Military Hospital of Santiago. The analytical process was conducted through the fully automated LAB CELL platform (Siemens) interfaced with the Advia 2120, Advia 1800 and Advia Centaur XP systems.

For the haemogram, the sample was stored in a BD Vacutainer in an EDTA tube and processed in an Advia 2120 through flow cytometry, optical laser and impedance. For the purpose of this study, the analysis comprised the number of red cells (RBC), haemoblobin (Hb), haematocrit (Hct) and mean corpuscular volume (MCV).

To measure the Cortisol and Total Testosterone (TT), the study used a BD Vacutainer with gel separator and coagulation activator and it was processed in an Advia Centaur XP by chemiluminescence. For the Free Testosterone (FT), the study used a BD Vacutainer with gel separator and coagulation activator. It was processed in an Immulite 2000 (Siemens) by radioimmunoassay (gamma counter).

Immediately after drawing the blood samples, these were sent to the clinical laboratory at the Military Hospital of Santiago, transported by personnel from the aforementioned laboratory in compliance with the regulations for the transport and storage of biological fluids.

An initial atmospheric pressure of 663 mmHg[23] was calculated and the oxygen saturation level ($Sa0_2$) was measured with a portable device (Nonin CMS50D, USA, 2014). Weight was measured with a Tanita scale (Tanita Ironman BC1500, Japan, 2015) and the tympanic temperature was taken before and after the march using an infrared thermometer. (Boeringher, Germany, 2015).

Statistical analysis

The data are presented as mean and standard deviation (SD) and were analysed using the Statistical Package for the Social Sciences 15.0 software (SPSS Inc, USA). For each analysis, the normality of distribution was tested using the Shapiro-Wilk test. The median and standard deviation were calculated for each measurement. In order to determine whether there were significant differences between the pre and post tests, the Student's paired t-test was applied to the variables with normal distribution and the Wilcoxon test to the not normally distributed variables. In all cases, we considered a confidence level of 95% (value $p < 0.05$).

Additionally, the difference between variables was valued by calculating the effect size (ES) through Cohen's d-test[24]. The d values were considered as: very small (d<0.1); small (d = 0.1 to 0.2); moderate (d = 0.21 to 0.5); large (d = 0.51 to 0.8) and very large (d > 0.8).

Results

Table 1 shows the data for the variables analysed and corresponding to the PRE and POST samples, together with the values for the Cohen's d test and the effect size.

All the changes were significant, except for the SaO_2 and the cortisol.

Discussion

The main contribution of this study is that, following a winter march with combat equipment, at a low altitude and with a difference in height of 746 m, the soldiers of a mountain operations unit showed a significant drop in the plasma values of FT and TT, with no changes in the cortisol values.

With regard to the cortisol, changes have been reported due to the effect of height, but only at moderate or high altitudes and so that it appears that the exposure process is the factor determining the behaviour of cortisol at these altitudes. When exposure is acute, cortisol has been observed to increase[7-9,14] however, with gradual exposures, no changes are observed in cortisol levels[11]. When physical exercise is performed at these heights, following acute exposure, a drop is reported in previously high cortisol levels, for saliva[12] and blood[10] alike. At low altitudes (such

Table 1. Measurement and standard deviation (SD) of weight, $Sa0_2$, temperature, cortisol (C), free testosterone (FT) and total (TT), FT/C ratio, RBC, haemoglobin, haematocrit and mean corpuscular volume (MCV).

Variable	Pre-test	Post-test	d	ES
Weight (kg)	75.2±7.6	74.1±7.58*	0.15	Small
Sa02 (%)	97.4±2.99	97.1±2.7	0.11	Small
Temperature (°C)	35.5±0.43	35.9±0.36*	1.02	Very large
Cortisol (uM/L)	0.75±0.12	0.72±0.17	0.26	Moderate
FT (uM/L)	43.4±11.5	24.7±14.1*	1.84	Very large
TT (uM/L)	11.2±3.8	4.94±3.22*	2.31	Very large
FT/C Ratio	60.3±19.7	36.4±22.3*	1.44	Very large
RBC (M/uL)	5.11±0.24	5.05±0.23*	0.26	Moderate
Haemoglobin (M/uL)	15.2±0.73	14.9±0.76*	0.42	Moderate
Haematocrit (°C)	45.3±1.9	43.5±1.98*	0.89	Very large
MCV (uM/L)	88.7±2.22	86.4±2.3*	1.01	Very large

*p<0.05; ES: effect size calculated with Cohen's d: d <0.1 (ES very small); d = 0.1 to 0.2 (ES small), d = 0.21 to 0.5 (ES moderate), d = 0.51 to 0.8 (ES large) and d > 0.8 (ES very large).

as those forming part of this study) no effects on cortisol have been reported in the literature.

On the other hand, the cortisol response to exercise is extremely variable, depending on the time of day, the type of exercise and the accumulated fatigue (as well as other factors) and it could therefore either increase, decrease or stay the same[25].

Therefore, given that the low altitude in itself has no effect on cortisol, the fact that there were no variations of cortisol in our group would indicate (in general) that the specific task performed would not have involved a stress stimulus that was sufficiently intense to raise the levels of cortisol in response to the same. However, this explanation is somewhat inconsistent with the effort that these soldiers had to make, as part of their training, taking into account that the march was made at a considerable average speed for the equipment that they were carrying and that it took place at night, at low temperatures and with a difference in height of close to 750 m.

With regard to the decrease observed in testosterone values, the normal response at high altitudes is not clear in the literature, if there actually is an effect that is directly related to altitude. Most of the studies consulted determine testosterone levels during training sessions in high mountain areas[15] or else the long term effect of exposure to high altitudes[4,11,26]. In any case, we have found no effects reported in the literature attributable to low altitudes such as those in this study.

On the other hand, with regard to physical exercise, a large number of works published use values of salivary testosterone with highly varied results, which agrees with the meta-analysis by Hayes, *et al.*[27] which found that the effects are highly dependent on the type of exercise, the study design and the sampling time. Another recent review[28] reveals that high-intensity exercise produces a reduction in the activity of the hypothalamic–pituitary–gonadal axis with the subsequent decrease in testosterone levels, while it finds that the data published for moderate intensity exercises are inconsistent. Pursuant to this review, our data would be more coherent with the work load that this task represents and with the fatigue level experienced by the subjects.

The FT/C ratio shows a marked decrease with values similar to those found in athletes under high work load or overexertion conditions[27,29,30].

With regard to the parameters for the red blood cells, all of these (RBC, Hb, Hct, and MCV) showed significant decreases after the march and with a moderate ES for RBC and Hb and very large for Hct and MCV (Table 1). In this section, the existing data also support the idea that these altitudes do not induce haematological changes by themselves. Thus, Rietjens[31], following the 3-year monitoring of high level triathletes, found that altitudes of at least 2,000 m are required in order to attribute haematological changes to altitude, and works frequently report these changes at higher altitudes[32,33]. In our opinion, the changes in the red blood count could be conditioned by a haemodilution effect that has already been reported for marathon runners[34]. With regard to the decrease observed in the MCV, this agrees with what Sewchand already described in 198035 finding that any acute exposure to altitude would produce a decrease in MCV of between 12 to 14%.

The principal limitation of this study lies in the fact that we were unable to differentiate the effect of the physical work load from the additional stressors (such as cold, lack of sleep or accumulated fatigue) and from the possible effect of the change in altitude, although, based on the literature, this latter factor could reasonably be ruled out. A further limitation was the failure to control the fluid intake during the march, although this aspect is slightly mitigated by the fact that we monitored the level of dehydration through double weighing.

Conclusions

A winter march of soldiers from a mountain operations unit, carrying combat equipment, conducted at a low altitude and with a change of height of 746 m, produces a significant drop in their plasma Testosterone levels (free and total).

No changes in the cortisol levels were observed.

A significant reduction was detected in RBC, haemoglobin, haematocrit and MCV, that could be due to the effect of haemodilution.

Acknowledgements

To the Doctrine Division, School of Mountaineering and Military Hospital of the Chile Armed Forces. To Major Mario Pizani, Daniela Cárdenas, Reinaldo Rosas, Vjera Triantafilo and Cecilia Rojas.

Ethics Approval Committee: Military Hospital of Santiago N° CEC/23.06.2016/HOSMIL-DIVDOC.

Conflict of interest

The authors have no conflict of interest whatsoever.

Bibliography

1. Bergeron MF, Bahr R, Bartsch P, Bourdon L, Calbet JA, Carlsen KH, *et al.* International Olympic Committee consensus statement on thermoregulatory and altitude challenges for high-level athletes. *Br J Sports Med.* 2012;46(11):770-9.
2. Hall A, Evans K, Pribyl S. Cold injury in the United States military population: current trends and comparison with past conflicts. *J Surg Educ.* 2010;67(2):61-5.
3. Heil KM, Keenan AM. Athletic altitude training protocols and their application in preparation for mountainous operations. *J R Nav Med Ser*. 2014;100(1):65-9.
4. Muza SR. Military applications of hypoxic training for high-altitude operations. *Med Sci Sports Exerc.* 2007;39(9):1625-31.
5. He J, Cui J, Wang R, Gao L, Gao X, Yang L, *et al*. Exposure to hypoxia at high altitude (5380 m) for 1 year induces reversible effects on semen quality and serum reproductive hormone levels in young male adults. *High Alt Med Biol*. 2015;16(3):216-22.
6. Nieto C, Cárcamo M. Entrenamiento y evaluación de la capacidad física militar: revisión de la literatura. *Rev Esp Edu. Fís y Dep.: REEFD.* 2016;415:75-86.
7. Zaccaria M, Rocco S, Noventa D, Varnier M, Opocher G. Sodium regulating hormones at high altitude: basal and post-exercise levels. *J Clin Endocrinol Metab.* 1998;83(2):570-4.
8. Barnholt KE, Hoffman AR, Rock PB, Muza SR, Fulco CS, Braun B, *et al.* Endocrine responses to acute and chronic high-altitude exposure (4,300 meters): modulating effects of caloric restriction. *Am J Physiol Endocrinol Metab.* 2006;290(6):E1078-88.
9. Sutton JR, Viol GW, Gray GW, McFadden M, Keane PM. Renin, aldosterone, electrolyte, and cortisol responses to hypoxic decompression. *J Appl Physiol.* 1977;43(3):421-4.
10. McLean CJ, Booth CW, Tattersall T, Few JD. The effect of high altitude on saliva aldosterone and glucocorticoid concentrations. *Eur J Appl Physiol Occup Physiol.* 1989;58(4):341-7.
11. Benso A, Broglio F, Aimaretti G, Lucatello B, Lanfranco F, Ghigo E, *et al.* Endocrine and metabolic responses to extreme altitude and physical exercise in climbers. *Eur J Endocrinol.* 2007;157(6):733-40.

12. Woods DR, Davison A, Stacey M, Smith C, Hooper T, Neely D, *et al.* The cortisol response to hypobaric hypoxia at rest and post-exercise. *Horm Metab Res.* 2012;44(4):302-5.

13. Larsen JJ, Hansen JM, Olsen NV, Galbo H, Dela F. The effect of altitude hypoxia on glucose homeostasis in men. *J Physiol.* 1997;504 (Pt 1):241-9.

14. Richalet JP, Letournel M, Souberbielle JC. Effects of high-altitude hypoxia on the hormonal response to hypothalamic factors. *Am J Physiol Regul Integ. Comp Physiol.* 2010;299(6):R1685-92.

15. Wang RY, Tsai SC, Chen JJ, Wang PS. The simulation effects of mountain climbing training on selected endocrine responses. *Chin J Physiol.* 2001;44(1):13-8.

16. Gonzales GF. Hemoglobina y testosterona: importancia en la aclimatación y adaptación a la altura. *Rev. Per. de Med. Exp. y Sal. Púb.* 2011;28(1):92-100.

17. Adlercreutz H, Harkonen M, Kuoppasalmi K, Naveri H, Huhtamieni H, Tikkanen H, *et al.* Effect training on plasma anabolic and catabolic steroid hormones and their responses during physical exercise. *Int J Sports Med.* 1986;(7):27-8.

18. Schelling X, Calleja-González J, Terrados N. Variation in testosterone and cortisol with relation to mood state in professional basketball players. RICYDE. *Rev. Int. de Ciencias del Deporte.* 2013;9(34):342-59.

19. Tanskanen M, Kyröläinen H, Uusitalo A, Huovinen J, Nissilä J, Kinnunen H, *et al.* Serum sex hormone-binding globulin and cortisol concentrations are associated with overreaching during strenuous military training. *J Strength Cond Res.* 2011;25(3):787-97.

20. Martínez AC, Seco J, Tur JA, Abecia LC, Orella EE, Biescas AP. Testosterone and Cortisol Changes in Professional Basketball Players Through a Season Competition. *J Strength Cond Res.* 2010;24(4):1102-8.

21. Urhausen A, Gabriel H, Kindermann W. Blood hormones as markers of training stress and overtraining. *Sports Med.* 1995;20(4):251-76.

22. Vervoorn C, Vermulst LJM, Boelens-Quist AM, Koppeschaar HPF, Erich WBM, Thijssen JHH, *et al.* Seasonal changes in performance and free testosterone: cortisol ratio of elite female rowers. *Eur J Appl Physiol.* 1992;64(1):14-21.

23. Hematy Y, Setorki M, Razavi A, and Doudi M. Effect of Altitude on some Blood Factors and its Stability after Leaving the Altitude. *Pak J Biol Sci*. 2014;17(9):1052-7.

24. Cohen, J. Statistical power analysis for behavioral sciences. Hillsdale, NJ: LEA; 1988.p.412-4.

25. Suárez AM, Javierre C, Ventura JL, Garrido E, Barbany JR, Segura R. Interindividual plasma cortisol differences in the response to one hour of aerobic exercise with inserted supramaximal workloads. *Apunts.* 2007;42(153):3-62.

26. Okumura A, Fuse H, Kawauchi Y, Mizuno I, Akashi T. Changes in male reproductive function after high altitude mountaineering. *High Alt Med Biol.* 2003;4(3):349-53.

27. Hayes LD, Grace FM, Baker JS, Sculthorpe N. Exercise-induced responses in salivary testosterone, cortisol, and their ratios in men: a meta-analysis. *Sport Med.* 2015;45(5):713-26.

28. Matos B, Howl J, Ferreira R, Fardilha, M. Exploring the effect of exercise training on testicular function. *Eur. Jour. of Appl. Phy*. 2018;1-8.

29. Gonzalez Jurado JA, De Teresa C, Molina E, Guisado R, Naranjo J. Efecto del Phlebodium Decumanum sobre los cambios en niveles plasmáticos de testosterona y cortisol inducidos por el ejercicio en sujetos no entrenados. *Rev. Med. de Chile.* 2009;137:497-503.

30. Di Blasio A, Izzicupo P, Tacconi L, Di Santo S, Leogrande M, Bucci I, *et al.* Acute and delayed effects of high intensity interval resistance training organization on cortisol and testosterone production. *The Jour. Of Sport. Med. And Phy. Fit*. 2016;56:192-9.

31. Rietjens GJM, Kuipers H, Hartgens F, Keizer HA. Red blood cell profile of elite olympic distance triathletes. A three-year follow-up. *Int J Sports Medicine.* 2002;23(6):391-6.

32. Heinicke K, Heinicke I, Schmidt W, Wolfarth B. A three-week traditional altitude training increases hemoglobin mass and red cell volume in elite biathlon athletes. *Int J Sports Med.* 2005;26(5):350-5.

33. Hematy Y, Setorki M, Razavi A, Doudi M. Effect of altitude on some blood factors and its stability after leaving the altitude. *Pak J Biol Sci.* 2014;17(9):1052-7.

34. Traiperm N, Gatterer H, Burtscher M. Plasma electrolyte and hematological changes after marathon running in adolescents. *Med. and scie. in spor. and exe*. 2013;45(6):1182-7.

35. Sewchand LS, Lovlin RE, Kinnear G, Rowlands S. Red blood cell count (RCC) and volume (MCV) of three subjects in a hypobaric chamber. *Aviat Space Enviro Med.* 1980; 51(6):577-8.